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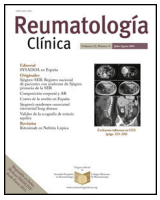

Original Article

# Anti-RNA polymerase III antibodies in systemic sclerosis: Multicentric study from Argentina☆

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ABSTRACT

*Objective:* To describe the frequency of anti-RNA polymerase III antibody in patients with Systemic Sclerosis (SSc) of a group of healthcare centres from Argentina and to explore differences among patients with positive and negative anti-RNA polymerase III antibody.
*Patients and methods:* Data from clinical records, anamnesis and physical examination were collected from 135 patients with SSc (ACR/EULAR 2013). A serum sample from each patient was obtained for the detection of anti-RNA polymerase III IgG antibodies by ELISA.
*Results:* In all, 97.8% were women and the median age at diagnosis was 53 years (range 12–87), 77.7% had limited cutaneous SSc (lcSSC), 19,3% patients had diffuse cutaneous SSc (dcSSC) and 2.9% had scleroderma sine scleroderma. The 67.5% of the patients were from a Mestizos or Amerindian ethnic group. Anti-RNA polymerase III was positive in 5.9% of the patients. In 36 patients, the anticentromere (ACA) and anti-Scl70 antibodies were negative; anti-RNA polymerase III was positive in 16.7% of these 36 patients. Pitting scars and pulmonary artery hypertension were more frequent in anti-RNA polymerase III positive patients who were also older at diagnosis. No association with gastric antral vascular ectasia was found. The only patient with scleroderma renal crisis was anti-RNA polymerase III positive.
*Conclusions:* Anti-RNA polymerase III frequency found in this study was one of the lowest reported, which could be related to the predominance of the Amerindian and Mestizo ethnic group. It is possible that the detection of anti RNA polymerase III allows better classification of SSc patients, to know their prognosis and to improve their follow-up, therefore more studies are needed.



## Anticuerpos anti-RNA polimerasa III en esclerosis sistémica: estudio multicéntrico de Argentina

*Palabras clave:*
Esclerosis sistémica
Autoanticuerpos
Anti-RNA polimerasa III

RESUMEN

*Objetivo:* Describir la frecuencia del anticuerpo anti-RNA polimerasa III positivo en pacientes con esclerosis sistémica (ES) de un grupo de centros asistenciales de Argentina, y explorar las diferencias entre pacientes con anti-RNA polimerasa III positivo y negativo.

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*Pacientes y métodos:* Se recopilaron datos de las historias clínicas, anamnesis y exámenes físicos de 135 pacientes con ES (ACR/EULAR 2013), de los cuales se obtuvo una muestra de suero para la detección de anti-RNA polimerasa III IgG por ELISA.
*Resultados:* El 92,6% fueron mujeres, la mediana de edad al diagnóstico fue de 53 años (rango 12–87), el 77,7% tenía ES limitada, el 19,3% tenía ES difusa y el 2,9% con ES *sine* esclerodermia. El 67,5% de los pacientes pertenecían a la etnia mestiza o amerindia. La frecuencia de anti-RNA polimerasa III positivo fue del 5,9%. En 36 pacientes los anticuerpos anticentrómero (ACA) y anti-Scl70 fueron negativos; el anti-RNA polimerasa III fue positivo en el 16,7% de estos 36 pacientes. El grupo de pacientes con anti-RNA polimerasa III positivo tuvo una mayor frecuencia de cicatrices puntiformes e hipertensión arterial pulmonar, y una mayor edad al diagnóstico. No se encontró asociación con ectasia vascular gástrica antral. La única paciente con crisis renal esclerodérmica, fue anti-RNA polimerasa III positiva.
*Conclusiones:* La frecuencia de anti-RNA polimerasa III encontrada en este estudio fue una de las más bajas reportadas, lo cual podría estar relacionado con el predominio de la etnia mestiza y amerindia. Si bien se necesitan más estudios, es posible que la detección del anti-RNA polimerasa III permita clasificar mejor a los pacientes con ES, conocer su pronóstico y mejorar el seguimiento.



## Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease that affects the connective tissue, characterised by inflammatory changes, excessive fibrosis of the skin and internal organs, microvascular disturbances and autoimmunity.[1] Ninety percent of patients with SSc have antinuclear antibodies (ANA)[2] and specific antibodies such as anti-topoisomerase1 (anti-Scl70), anti-RNA polymerase III, anti-U3-RNP, anticentromere (ACA) and anti-Th/To.[2,3] The presence of specific antibodies classifies patients into groups with different frequency of organ involvement, clinical course and disease prognosis.[3–5] Anti-Scl70 and anti-U3-RNP are associated with the diffuse cutaneous form, while the presence of ACA and anti-Th/To are associated with the limited cutaneous form.[2,3] The patients' serological profile is not altered by treatment or the course of the disease.[3,4]

The anti-RNA polymerase III antibody has been described in patients with SSc, especially diffuse SSc and scleroderma renal crisis (SRC), a severe complication affecting up to 5% of SSc patients in the first years of the disease.[2–7] Anti-RNA polymerase III is also associated with the presence of gastric antral vascular ectasia (GAVE),[8] and with some types of cancer in patients with SSc.[9–11]

The frequency of positive anti-RNA polymerase III in SSc varies widely in different series. The frequency was 25% in a U.S. cohort of patients, whereas it was 4% in a French cohort. The frequency of this antibody has been little studied in the Latin American population. A frequency of 1.4% was found in a cohort of mixed-race Mexican patients. No reports were found in the Argentine population.

The aims of this study were to describe the frequency of positive anti-RNA polymerase III antibody in patients with SSc from a group of healthcare centres in Argentina, and to explore the differences in clinical and demographic characteristics between patients with positive and negative anti-RNA polymerase III antibodies.

## Patients and methods

### *Patients*

Patients of both sexes, over 18 years of age, who met the 2013 American College of Rheumatology and European League Against Rheumatism classification criteria (ACR/EULAR 2013)[14] for SSc were included in the study. The project was approved by the Teaching and Ethics Committee. All participants signed an informed consent form.

The patients came from 7 healthcare centres in Argentina (4 in the Autonomous City of Buenos Aires, 2 in the province of Buenos Aires and one in the province of Santa Fe) that form part of the Scleroderma Study Group of the Argentine Society of Rheumatology (GESAR *Esclerodermia*).

### *Clinical data*

With information from the interview, physical examination and clinical history, the rheumatologists completed a form for each patient in which demographic data – including ethnicity as defined by the GLADEL group[15] – personal history of hypertension, dyslipidaemia, smoking, primary biliary cholangitis (PBC), and cancer were recorded. The type of SSc, disease progression – defined as the time from onset of the first symptom attributable to SSc until the time of completing the form – and the clinical manifestations of the disease were recorded.

### *Clinical manifestations*

Clinical involvement was categorised according to the classification of organs and systems of the SSc severity scale.[16] The clinical manifestations that were considered to define the involvement of different organs and the studies used for the diagnosis of each complication are detailed below:

- *Vascular involvement:* report of Raynaud's phenomenon, pitting scars (defined as a lesion with central depression, surrounded by hyperkeratosis on the finger pads), ulcers or digital necrosis.
- *Skin involvement:* presence of sclerosis assessed by modified Rodnan skin score,[17] telangiectasias and/or calcinosis — documented by clinical findings or imaging.
- *Joint involvement:* presence of arthritis and/or tendon friction rubs ("leather crepitus" on palpation of the hands).[18]
- *Muscle involvement:* detection of muscle weakness on physical examination, or due to increased muscle enzymes (creatine phosphokinase [CPK] and/or aldolase) in complementary studies, or due to myopathic changes on electromyogram.
- *Digestive tract involvement:* Gastrointestinal tract involvement: included swallowing involvement assessed by video swallow study; oesophageal disturbances detected by digestive videoendoscopy, oesophageal manometry or barium swallow; gastric involvement defined by the presence of GAVE-type or "watermelon stomach" involvement on panendoscopy (gastroscopy); small bowel and colon involvement defined by the presence of malabsorption syndrome, diarrhoea, constipation or malnutrition with or without supplementary enteral nutrition requirement.
- *Cardiac involvement:* included the presence of pericarditis, impaired systolic and/or diastolic function, both detected by

Doppler echocardiogram; or conduction system disturbances detected by electrocardiogram or Holter monitoring.
- *Pulmonary involvement:* pulmonary involvement was subject to the presence of interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH). ILD was defined as the presence of a forced vital capacity <70% and a forced expiratory volume in the first second >80% on the functional respiratory examination with plethysmography; or the presence of interstitial fibrosis or ground-glass changes on radiography or high-resolution computed tomography. PAH was defined as a pulmonary artery systolic pressure by echocardiogram >45 mmHg and a mean pulmonary artery pressure by right heart catheterisation >25 mmHg.
- *Renal involvement:* the SRC report was considered, defined by a history of abrupt-onset hypertension, microangiopathic anaemia and rapidly progressing oliguric renal failure.

### *Antibody testing*

The results of ANA testing by indirect immunofluorescence assay (IFA), of ACA and anti-Scl70 were obtained if available in the clinical histories.

A serum sample was taken from each patient to detect anti-RNA polymerase III isotype IgG antibodies, and sent to and processed in the immunology laboratory of the Instituto de Investigaciones Médicas Dr. Alfredo Lanari. The antibody was measured by ELISA using a commercial semi-quantitative kit (Quanta Lite RNA Pol III, INOVA®, San Diego, USA), currently available for healthcare purposes, which uses a purified recombinant immunodominant fragment of RNA polymerase III as the antigenic target.[19,20] The cut-off value of the assay is 20U. Antibody values between 20–39U were considered weak positive, values between 40–80U moderate positive, and values greater than 80U strong positive. According to the manufacturer's information, the clinical specificity of anti-RNA polymerase III ELISA is greater than 99% and the clinical sensitivity is 23%.[21]

### *Statistical analysis*

The results are reported as mean ± standard deviation or median (range: minimum-maximum) for quantitative variables, and as frequencies and percentages for qualitative variables.

To explore differences in clinical and demographic characteristics between anti-RNA polymerase III positive and negative patients, the group of anti-RNA polymerase III positive patients was compared with 3 different groups of anti-RNA polymerase III negative patients considered the control groups: a) a group consisting of ACA positive patients only; b) a group consisting of anti-Scl70 positive patients; and c) a group of anti-Scl70, ACA and anti-RNA polymerase III negative patients.

The $\chi^2$ test or Fisher's test were used to compare proportions. Numerical variables were compared using the test of variance or the Kruskal–Wallis test. A 2-tailed p-value < .05 was considered significant. STATA v.11.0 was used for the statistical analysis of the data.

## Results

### *Description of the sample*

Data from 135 patients with SSc (125 females and 10 males) were analysed with a median age at diagnosis of 53 years (range:12–87); 26 patients were Caucasian (19.2%), 72 were of mixed race (53.3%), 19 were Amerindian (14.2%), and the ethnicity of the remaining 18 patients was not recorded (13.3%). A history of dyslipidaemia was recorded in 44 patients (32.6%), hypertension in 54 (40%) and smoking in 35 (25.9%). The clinical form of SSc was limited in 105 patients (77.7%), diffuse in 26 (19.3%), and SSc sine scleroderma in 4 (2.9%). The median disease progression was 5.5 years (range: 0–41). Table 1 describes the comorbidities and clinical manifestations found in the patients with diffuse and limited SSc. The median Rodnan skin score was 24.5 in diffuse SSc (range: 0–36) and 4 in limited SSc (range: 0–30).

**Table 1**
Clinical characteristic of the patients with diffuse and limited SSc.

| Clinical characteristics | Diffuse SSc (n = 26) | Limited SSc (n = 105) |
|---|---|---|
| *Comorbidities* | | |
| Arterial hypertension | 10 (38) | 42 (40) |
| Dyslipidaemia | 8 (31) | 34 (32) |
| Smoking | 7 (27) | 27 (26) |
| Primary biliary cholangitis | 1 (4) | 8 (8) |
| Cancer | 0 (0) | 3 (3) |
| *Vascular involvement* | | |
| Raynaud's phenomenon | 26 (100) | 102 (97) |
| Pitting scars | 11(42) | 28 (27) |
| Digital ulcers | 6 (23) | 24 (23) |
| Digital necrosis | 3 (11) | 5 (5) |
| *Skin involvement* | | |
| Sclerodactyly | 17 (65) | 59 (56) |
| Calcinosis | 1(4) | 8 (8) |
| Telangiectasias | 10 (38) | 74 (70) |
| *Joint involvement* | | |
| Tendon friction rubs | 1 (4) | 4 (4) |
| Arthritis | 7 (27) | 19 (18) |
| *Muscular involvement* | | |
| Myositis | 4 (15) | 3 (3) |
| *Gastrointestinal involvement* | | |
| Oesophagus | 18 (69) | 67 (64) |
| GAVE[a] | 1(4) | 2 (2) |
| Bowel | 2 (8) | 13 (12) |
| *Cardiac involvement*[b] | | |
| Impaired conduction system | 4 (15) | 13 (12) |
| *Pulmonary involvement* | | |
| Pulmonary arterial hypertension | 6 (23) | 10 (10) |
| Interstitial lung disease | 17 (65) | 25 (24) |
| *Renal involvement* | | |
| Scleroderma renal crisis | 1 (4) | 0 (0) |

The values are expressed as number of patients and the values in percentages (%) are shown in brackets.
GAVE: gastric antral vascular ectasia; SSc: systemic sclerosis.
[a] A panendoscopy report was available for 126 patients.
[b] None of the patients presented systolic and/or diastolic dysfunction.

All 4 patients with SSc sine scleroderma had Raynaud's phenomenon; digital necrosis was observed in 3 and arthritis in one. Two of the 4 patients had telangiectasias and oesophageal and pulmonary involvement.

### *Frequency of antibodies*

ANAs were positive in 93.3% of the patients studied (126/135). ACA was positive in 57.7% (75/130) and anti-Scl70 in 13.2% of the patients (17/129).

Anti-RNA polymerase III was positive in 8 out of 135 patients, a frequency of 5.9%. Two anti-RNA polymerase III positive patients were also ACA positive.

A higher frequency of positive ACA was observed in limited SSc (71.3 versus 4%) and of anti-Scl70 in diffuse SSc (52 versus 3%). Anti-RNA polymerase III was positive in 3 out of 26 patients (11.5%) with diffuse SSc, and in 3 out of 10 patients (4.8%) with limited SSc. Table 2 shows the frequency of positive antibodies according to the type of SSc.

**Table 2**
Frequency of positive antibodies in patients with SSc.

| Positive antibody | Diffuse SSc | Limited SSc | Scleroderma *sine* scleroderma |
|---|---|---|---|
| ANA | 23/26 (88.4) | 101/105 (96.2) | 2/4 (50) |
| ACA[a] | 1/25 (4) | 72/101 (713) | 2/4 (50) |
| Anti-Scl70[b] | 13/25 (52) | 3/100 (3) | 1/4 (25) |
| RNA Pol III | 3/26 (11.5) | 5/105 (4.8) | 0 (0) |

The results are reported as the number of positives/total results available. The percentage values (%) are shown in brackets. ACA: Anticentromere antibodies; ANA: antinuclear antibodies; RNA Pol III: anti-RNA polymerase III; SSc: systemic sclerosis.

[a] ACA result not available for 5 patients.

[b] Anti-Scl70: result not available for 6 patients.

Both ACA and anti-Scl70 were negative in 36 patients (11 patients with diffuse SSc, 24 with limited SSc and one with *sine* scleroderma). Of these patients, 16.7% (6 of 36) were anti-RNA polymerase III positive (3 with diffuse SSc and 3 with limited SSc) and the median antibody titre was 104U (range: 87–117).

In 2 patients with limited SSc, anti-RNA polymerase III coexisted with positive ACA and the titres were 43 and 44U, respectively. All 4 patients with limited SSc and tendon friction rubs were anti-RNA polymerase III negative.

*Comparison of anti-RNA polymerase III positive and negative groups*

Table 3 shows the comparison of demographic and clinical characteristics between the 4 groups of patients: the anti-RNA polymerase III positive group, the anti-Scl70 positive group, the ACA positive group only, and the anti-RNA polymerase III, ACA and anti-Scl70 negative group.

The anti-RNA polymerase III positive group was older at diagnosis, although this did not reach statistical significance. No significant differences were observed in terms of sex, years of disease progression or mixed ethnicity among the 4 groups of patients studied. However, the highest frequency of mixed ethnicity was found in the anti-RNA polymerase III, anti-Scl70 and ACA negative group.

The anti-RNA polymerase III positive group had a lower proportion of patients with diffuse SSc than the anti-Scl70 positive group, which was statistically significant.

The anti-RNA polymerase III positive group had the highest proportion of patients with pitting scars, although this did not reach statistical significance. Similar Rodnan skin scores were observed in the 4 patient groups, although the ACA-positive group showed the lowest median value.

The group of anti-RNA polymerase positive patients had the highest proportion of pulmonary arterial hypertension (37.5%), but the difference was not statistically significant. This group also showed the lowest proportion of interstitial lung disease (12.5%), with statistically significant differences compared to the Scl70-positive group. The only patient with SRC was anti-RNA polymerase III positive. None of the anti-RNA polymerase III positive patients had GAVE or cancer.

## Discussion

SSc is characterised by specific antibodies directed against ribonuclear proteins, kinetochore proteins, and cellular enzymes such as topoisomerase and RNA polymerase.[1–5] RNA polymerases are a set of enzymes involved in the synthesis of messenger RNA or DNA transcription. There are 3 types of RNA polymerase (I, II and III) in the eukaryotic cells, each specialised in the synthesis of a specific RNA. Anti-RNA polymerase I and III antibodies are specific to SSc, while anti-RNA polymerase II antibodies are also present in patients with SLE and overlap syndrome.[22]

The frequency of positive anti-RNA polymerase III is different according to the technique used and the geographical origin of the population studied. Immunoprecipitation is the gold standard method to detect anti-RNA polymerase III, which is not always available in daily practice.[22,23] Several studies have measured anti-RNA polymerase III using this technique and reported frequencies of 4% in a cohort from France,[12] 12% in a cohort from the UK,[24] and 25% in a cohort from the USA.[12] The IFA technique in Hep-2 cells has also been used as a strategy to detect anti-RNA polymerase III by several authors.[23,25] However, there is no consensus on a specific fluorescence pattern produced by this antibody.[4,22,23,25] Using the commercial ELISA assay to measure anti-RNA polymerase III antibodies in serum, highly variable frequencies were also reported among different cohorts, at 6% in Japan,[5] 9.4% in France[27] and up to 19% in Canada,[20] among others.

The present series of 135 patients from a group of healthcare centres in Argentina included 67.5% of patients of mixed and Amerindian ethnicity. The antibody was measured by ELISA, which detected positive anti-RNA polymerase III in 5.9% of patients. This frequency is among the lowest compared to other cohorts. Sobanski et al.,[27] despite the heterogeneity of the studies included in a meta-analysis of 8437 adult patients with SSc, estimated an overall prevalence of anti-RNA polymerase III of 11% (95% CI 8–14). However, a study that included 139 mixed-race Mexican patients with SSc found an anti-RNA polymerase III frequency of 1.4%; only two patients had positive anti-RNA polymerase III (measured by ELISA), one with diffuse SSc and the other with limited SSc.[13] These data suggest that the frequency of anti-RNA polymerase III would be lower in cohorts that include mixed-race or Amerindian patients compared to cohorts with predominantly Caucasian patients. Although the lower frequency could be explained by environmental or genetic factors, one study describes a similar immunogenetic predisposition among Caucasian, African American and Hispanic individuals with SSc in Texas, although it finds significant sociodemographic, clinical, and serological differences in disease expression among the 3 ethnic groups.[26]

In the present study, in line with Cavazzana et al.,[28] the only serological marker was positive anti-RNA polymerase III in 16.7% of ACA-negative, anti-Scl70-negative SSc patients. Although further studies are needed to evaluate the usefulness of the antibody, the results suggest a possible benefit of measuring anti-RNA polymerase III in patients with SSc and other negative specific antibodies.[22–28]

Although some authors have posited that specific antibodies are mutually exclusive in SSc, in this series 2 patients had positive anti-RNA polymerase III and ACA. The anti-RNA polymerase III titres detected in these 2 patients were lower than those found in patients whose only positive antibody was anti-RNA polymerase III. Other papers also describe a small number of patients in whom positive anti-RNA polymerase III coexists with anti-U3-RNP or anti-Scl70 or ACA25-28. There is still insufficient information available to assess the clinical significance of the coexistence of these antibodies.

Although the frequency of specific antibodies varies according to the type of SSc and the cohort studied, it has been reported that anti-RNA polymerase III is more frequent in patients with diffuse SSc. In this study, in line with the literature, the frequency of anti-RNA polymerase III was 4.8% in patients with limited SSc and 11.5% in diffuse SSc.

In this study, we found no significant differences in terms of sex, mixed ethnicity, and years of disease progression between the anti-RNA polymerase III-positive patients and the patients in groups with different antibody profiles. It was observed that, although not statistically significant, anti-RNA polymerase III-positive patients were older at diagnosis of the disease.

The patients with anti-RNA polymerase III also had a higher frequency of pitting scars. Pitting scars are considered a clinical

**Table 3**
Comparison of characteristics in anti-RNA polymerase III positive and negative patient groups.

| Variables | Anti-RNA polymerase III, anti-Scl70 and ACA negative (n = 30) | ACA positive (n = 73) | Anti-Scl70 positive (n = 17) | Anti-RNA polymerase III positive (n = 8) | p-Value |
|---|---|---|---|---|---|
| Female sex, n (%) | 28 (93.3) | 68 (93.2) | 14 (82.3) | 8 (100) | .412 |
| Median age at diagnosis (range) | 50 (12–78) | 54 (15–87) | 57 (24–75) | 63 (53–67) | .108 |
| Median years of disease progression (range) | 5 (0–41) | 5 (0–31) | 3 (0–9) | 6 (0–7) | .508 |
| Mixed ethnicity, n (%) | 22 (733) | 35 (47.9) | 7 (41.2) | 4 (50.0) | .076 |
| Diffuse form, n (%) | 8 (26.7) | 1 (1.4) | 13 (76.5)[a] | 3 (37.5) | .000 |
| Pitting scars, n (%) | 9 (32.1) | 20 (28.2) | 4 (26.7) | 6 (75.0) | .071 |
| Median Rodnan skin score (range) | 9 (0–36) | 4 (0–31) | 9 (0–30) | 9 (1–25) | .256 |
| Pulmonary arterial hypertension, n (%) | 3 (10.0) | 7 (9.6) | 3 (17.6) | 3 (37.5) | .117 |
| Interstitial lung disease, n (%) | 12 (40.0) | 17 (23.3) | 10 (58.8)[a] | 1(12.5) | .015 |
| SRC, n (%) | 0 (.0) | 0 (.0) | 0 (.0) | 1 (12.5) | .063 |
| GAVE, n (%) | 1 (3.3) | 1 (1.4) | 1 (5.9) | 0 (.0) | .456 |
| Cancer, n (%) | 2 (6.7) | 1 (1.4) | 0 (.0) | 0 (.0) | .347 |

ACA: anticentromere; anti-Scl70: anti-topoisomerase1; GAVE: gastric antral vascular ectasia; n: number of patients; %: percentage; SRC: scleroderma renal crisis.
[a] Group showing a statistically significant difference when compared to the anti-RNA polymerase III positive group.

expression of progressive ischaemic damage associated with Raynaud's phenomenon, but not always associated with more severe disease.

Although different studies describe that patients with positive anti-RNA polymerase III experience more rapid and severe skin thickening,[3,28] in this study no significant difference was found in median Rodnan skin score between the anti-RNA polymerase III positive group, the anti-Scl70 positive group and the anti-RNA polymerase III, ACA and anti-Scl70 negative group. Although the difference did not reach statistical significance, the ACA-positive group had the lowest median Rodnan score; this could be explained by the association of ACA with the limited form of the disease.

The presence of SSc-specific antibodies has already been linked to the development of pulmonary complications. Interstitial lung disease is more frequent in patients with diffuse skin involvement and anti-Scl70 positive, and pulmonary arterial hypertension with limited skin involvement and presence of ACA.[2,3] The positive anti-RNA polymerase III group had a higher frequency of pulmonary arterial hypertension than interstitial lung disease, a predictable result, as other series describe a lower frequency of severe pulmonary disease in patients with diffuse SSc and positive anti-RNA polymerase III.[3]

Gastrointestinal tract involvement is a frequent complication in SSc; GAVE-type involvement is described in 5.7% of patients,[29] and anti-RNA polymerase III is proposed as a predictive antibody for its diagnosis.[8] Cavazzana et al.[28] reported GAVE-type involvement of 16.7% in patients with positive anti-RNA polymerase III. However, in the present study, all 3 patients with this condition were anti-RNA polymerase III negative.

Several authors flag up the relationship between SRC and positive anti-RNA polymerase III in diffuse SSc. In line with the literature, the only patient in this series with scleroderma renal crisis had diffuse SSc and was anti-RNA polymerase III positive.[1–5,27,28]

Positive anti-RNA polymerase III was also associated with the development of cancer at the time the SSc was diagnosed.[9–11] In this study, only 3 patients had a history of cancer, and all were anti-RNA polymerase III negative.

A limitation of this study is that patients with SSc were included regardless of whether the diagnosis was recent. This resulted in a heterogeneous sample, with a median progression of 5.5 years, therefore it was not possible to establish the temporal relationship between the onset of the disease, the presence of some manifestations and the history of cancer. In this series, only 8% calcinosis was found in the group of patients with limited SSc, a low frequency compared to the 24.7% reported by Valenzuela et al.[30] in 5218 patients with SSc. However, calcinosis is considered a late manifestation of the disease (over 7.5 years), which possibly explains the lower frequency in this sample. Tendon friction rubs were another manifestation that proved infrequent. Tendon friction rubs are a physical examination finding, which usually precede skin thickening and can be considered a sign of poor prognosis, usually associated with the diffuse form of the disease.[31] It is striking that, in this study, four patients with tendon friction rubs belonged to the limited SSc group, but no follow-up data are available for these patients to ascertain whether these were early forms of diffuse SSc.

In conclusion, this multicentre study that included patients from 7 healthcare centres in Argentina, whose anti-RNA polymerase III antibodies were tested with a commercial ELISA assay, found a frequency of positive results of 5.9%. This is one of the lowest frequencies reported, which suggests that the frequency of anti-RNA polymerase III could be lower in cohorts that include mixed-race or Amerindian patients, compared to those with a predominance of Caucasian patients. Of the ACA and anti-Scl70 negative patients, 16.7% were anti-RNA polymerase III positive. It is possible that detecting anti-RNA polymerase III may enable better classification, prognosis, and follow-up in these patients. Further studies involving larger numbers of patients are needed to analyse the clinical impact of anti-RNA polymerase III in SSc.

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## Conflict of interests

The authors have no conflict of interests to declare.

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