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Original article

Autoantibodies in systemic sclerosis and their clinical correlation in patients from a Midwestern region of Brazil

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ABSTRACT

Introduction: Systemic sclerosis (SSc) is a connective tissue disease of autoimmune nature characterized by the triad of vascular injury, autoimmunity (cellular and humoral) and tissue fibrosis. Autoantibodies do not seem to be simply epiphenomena, but are involved in disease pathogenesis. It is believed that the SSc-specific autoantibodies are responsible both for amplifying immune response and targeting cell types that are relevant in the pathophysiology of SSc.

Objectives: To correlate the profile of the following specific autoantibodies: anti-centromere (ACA), anti-topoisomerase I (topo I) and anti-RNA polymerase III (RNAP III) with clinical and laboratory manifestations were observed in 46 patients with SSc in the Midwest region of Brazil.

Methods: The occurrence of specific autoantibodies in 46 patients with SSc was investigated, correlating the type of autoantibody with clinical and laboratory manifestations found.

Results: Among all patients evaluated, we found a predominance of females (97.8%), mean age 50.21 years old, Caucasian (50%), limited cutaneous SSc (47.8%), time of diagnosis between 5 and 10 years (50%), and disease duration of 9.38 years. According to the specific autoantibody profile, 24 patients were ACA-positive (52.2%), 15 were positive for anti-topo I (32.6%), and 7 showed positive anti-RNAP III (15.2%). The anti-topo I autoantibody correlated with diffuse scleroderma, with greater disease severity and activity, with worse quality of life measured by the SHAQ index, with a higher prevalence of objective Raynaud's phenomenon and digital pitting scars of fingertips. The ACA correlated with limited scleroderma, with earlier onset of disease, as well as higher prevalence of telangiectasias. The anti-RNAP III correlated with diffuse scleroderma, with a higher occurrence of subjective Raynaud's phenomenon and muscle atrophy. There was no association between the positivity for anti-topo I, ACA and anti-RNAP III antibodies and other variables related to laboratory abnormalities, as well as Rodnan skin score and skin, vascular, musculoskeletal, gastrointestinal, cardiopulmonary and renal manifestations.

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Conclusions: The clinical subtype of the disease and some clinical manifestations in SSc may correlate positively with the presence of specific autoantibodies.

Autoanticorpos em esclerose sistêmica e sua correlação com as manifestações clínicas da doença em pacientes do Centro-Oeste do Brasil

Palavras-chave:
Autoanticorpos
Esclerose sistêmica
Antitopoisomerase I
Anticentrômero
Anti-RNA polimerase III

RESUMO

Introdução: a esclerose sistêmica (ES) é uma enfermidade do tecido conjuntivo de caráter autoimune caracterizada pela tríade de injúria vascular, autoimunidade (celular e humoral) e fibrose tecidual. Os autoanticorpos não parecem ser simplesmente epifenômenos, mas sim estarem envolvidos na patogênese da doença. Acredita-se que os autoanticorpos específicos da ES são responsáveis tanto pela amplificação da resposta imune quanto por alvejar os tipos celulares que são relevantes na fisiopatologia da ES.
Objetivos: correlacionar o perfil de autoanticorpos específicos (anti-SCL70, ACA, anti-POL3) com as manifestações clínicas e laboratoriais observadas em 46 pacientes com ES da região Centro-Oeste do Brasil.
Métodos: pesquisou-se a ocorrência de autoanticorpos específicos em 46 pacientes com diagnóstico de ES e correlacionou-se o tipo de autoanticorpo com as manifestações clínicas e laboratoriais encontradas.
Resultados: dentre todos os pacientes avaliados, encontrou-se predomínio feminino (97,8%), idade média de 50,21 anos, cor branca (50%), forma limitada da doença (47,8%), tempo de diagnóstico entre cinco e 10 anos (50%) e tempo de evolução da doença de 9,38 anos. De acordo com o autoanticorpo específico, 24 pacientes apresentavam ACA positivo (52,2%), 15 apresentavam positividade para anti-SCL70 (32,6%) e sete apresentavam anti-POL3 positivo (15,2%). O autoanticorpo anti-SCL70 se correlacionou com a forma difusa da doença, com maior gravidade e atividade da doença, com pior qualidade de vida medida pelo índice HAQ, com maior prevalência de fenômeno de Raynaud objetivo e microcicatrizes de polpas digitais. O ACA se correlacionou com a forma limitada da doença, com o início mais precoce da enfermidade, bem como com maior prevalência de telangiectasias nos pacientes. Já o anti-POL3 se correlacionou com a forma difusa da doença, com maior ocorrência de fenômeno de Raynaud subjetivo e de atrofia muscular. Para as demais variáveis relacionadas às alterações laboratoriais, bem como em relação ao escore cutâneo de Rodnan e às manifestações cutâneas, vasculares, musculoesqueléticas, gastrintestinais, cardiopulmonares e renais, não houve associação entre elas e a positividade para os anticorpos anti-SCL70, ACA e anti-POL3.
Conclusões: a forma clínica da doença e algumas manifestações clínicas na ES podem se correlacionar positivamente com a presença de autoanticorpos específicos.

Introduction

Systemic sclerosis (SSc) is a connective tissue disease of autoimmune nature, extremely heterogeneous in its clinical presentation, with involvement of multiple systems, and following a variable and unpredictable course.[1] Its etiology remains unknown, with a multifactorial cause being suggested, possibly triggered by environmental factors in a genetically predisposed individual.[2]

The hallmark of SSc is microvasculopathy, activation of fibroblasts and excessive collagen production.[3] It is a unique disease as it has features of three distinct pathophysiological processes; it consists of the triad of vascular injury, autoimmunity (cellular and humoral) and tissue fibrosis, leading to involvement of skin, in addition to several internal organs such as lungs, heart, gastrointestinal tract, among others.[3,4]

It is believed that the link between initial vascular involvement and the final consequence of the disease (tissue fibrosis) could be represented by autoimmunity. Circulating antibodies, alteration of immune mediators and infiltration of mononuclear cells in affected organs represent a positive argument for the hypothesis that dysfunction of the immune system leads to illness.[5,6]

It is described that highly specific antibodies can be detected in the sera of virtually all patients with SSc.[7] A review article by Zimmermann and Pizzichini highlights that specific antibodies represent one of the hallmarks of the disease and constitute the most evident expression of the

involvement of the humoral immune system in the genesis of SSc.[8] These autoantibodies have fundamental characteristics of a response triggered by the antigen, being mainly represented by: centromere antibodies (ACA), anti-DNA topoisomerase I (anti-topo I), and anti-RNA antibodies polymerase III (anti-RNAP III).[9–12]

Recent studies highlight the pathogenic potential of autoantibodies in SSc patients, suggesting that specific antibodies against fibroblasts, endothelial cells and receptors for platelet-derived growth factor (PDGF) can directly cause activation of fibroblasts and endothelial cells and contribute to tissue damage.[1,13,14]

There is evidence to support the idea that the complexity of the SSc seems to represent another collection of phenotypes compared to a single disease entity. Thus, these genetic associations may actually be related to distinct phenotypes in the SSc based on a pattern of autoantibodies.[15]

The region of the HLA genes is a clear example of genetic polymorphism in the development of SSc.[15] HLA association studies were very inconsistent when patients were grouped by race or ethnicity.[15,16] However, when patients with SSc were grouped according to the autoantibody profile, the findings were consistent across the different ethnic groups.[15] For example, HLA-DRB1*01–DQB1*0501 are more common in patients with ACA-positive SSc while haplotypes HLA-DRB1*11–DQB1*0301 have been associated with the positivity of anti-topo I antibodies.[16]

The mechanisms postulated for the development of autoantibodies in SSc patients include: molecular mimicry, chronic hyper-reactivity of B lymphocytes from intrinsic abnormalities of the cell and increased expression, or altered subcellular localization of potentially auto-antigenic peptides.[1] Some antibodies do not appear to be simply epiphenomena, but to be involved in disease pathogenesis,[17] possibly by amplifying the immune response and targeting cell types that are relevant in the pathophysiology of the disease.[18]

The importance of the study of autoantibodies in SSc lies in the fact that some of them have association with the disease and participate in the criteria proposed by LeRoy and Medsger for early diagnosis of the condition, which gives them considerable diagnostic value.[9,10,19] Moreover, they are associated with certain phenotypic traits of the disease, and are used to aid in the classification and characterization of two major disease subtypes: diffuse cutaneous SSc and limited cutaneous SSc.[10,20] Also, a close relationship between levels of anti-topo I and severity of skin involvement and global disease activity in SSc was observed, revealing a possible prognostic role.[10,21] In addition, significant correlations between the pattern of antibody profile presented and the therapeutic response have been reported.[22]

Objectives

To correlate the profile of specific autoantibodies (ACA, anti-topo I and anti-RNAP III) with clinical manifestations observed in 46 patients with SSc followed in a university center from the Midwest region of Brazil.

Methods

This is an observational study of cross-sectional design, with prospective analysis of patient data.

A random selection of 46 patients was carried out from a survey of the medical records of the Department of Rheumatology of the University Hospital of the School of Medicine of the Federal University of Mato Grosso do Sul (FMUFMS).

The patients were divided into three groups, according to the positivity of one of the specific autoantibodies (ACA, anti-topo I and anti-RNAP III).

Patients should meet the following inclusion criteria:

- Meet the 2013 new classification Criteria for SSc;[23]
- In cases of absence of skin thickening, they should meet the 2001 LeRoy and Medsger's criteria of early SSc;[24]
- They should have signed a Consent Form previously approved by the Research Ethics Committee of UFMS.
- Patients who presented with other associated infectious diseases or malignancies were excluded.

The socio-demographic and clinical information was obtained from the patient medical records and complemented with two interviews, within a time interval of 6 months. In the first appointment, demographic and clinical data were collected, including disease duration, year of diagnosis, modified Rodnan skin score,[25] autoantibodies test, thorough clinical examination and current treatment.

Disease duration was divided into two: total time in years of Raynaud's phenomenon (RP) before diagnosis of the disease (RP time) and total time in years of clinical manifestations of the disease after diagnosis, not considering RP (time without RP).

Specific data about Medsger's Severity Criteria[26] and Valentini's Criteria of Activity[27] were collected on specific formularies at baseline assessment and after 6 months. The Scleroderma Health Assessment Questionnaire (SHAQ)[28] was also collected in the initial patient assessment and on second assessment.

SHAQ is a measure of function in SSc, being a helpful tool for the assessment of physical functional disability[29] and the impact of the disease on patient's physical and mental well-being.[30] The objective was to correlate if the rate of disability measured by SHAQ would be higher in one of the three groups of patients with specific autoantibodies (ACA, anti-topo I and anti-RNAP III).

Sera properly frozen at −50 °C and stored at the Laboratory of the University Hospital of UFMS from previously selected patients was used for performing the research.

(a) For the examination of anti-centromere (ACA) – we used the indirect immunofluorescence technique and having HEp2 cells as substrate according to the criteria of the II Brazilian Consensus of antinuclear antibody in Hep-2 (2003) cells,[31] for the interpretation of results.
(b) For the examination of anti-DNA topoisomerase 1 (anti-topo I) – enzyme immunoassay technique was used,[21] being non-reactive if <20 units, weakly positive between 20 and 39 units, moderately positive between 40 and 80 units

and strongly positive if >80 units. A specific kit QUANTA Lite TM Scl-70 was used from Laboratory INOVA (INOVA Diagnostics, Inc., San Diego, CA, USA), following the manufacturer's specifications.

(c) Anti-RNA polymerase III (anti-RNAP III) antibody – examinations were performed in duplicate using ELISA technique, as previously described.[32] Values <20 units were considered negative, weakly positive if between 20 and 39 units, moderately positive if between 40 and 80 units and strongly positive if >80 units.

Statistical analysis

Comparison of patients with positive anti-topo I antibody, ACA or anti-RNAP III in relation to the quantitative variables evaluated in this study was performed by one-way ANOVA.

The chi-square test was used to assess the association between the results for the antibodies (ACA, anti-topo I and anti-RNAP III), with qualitative variables measured in this study. The results of the other variables were presented in the form of descriptive statistics or in the form of tables and graphs. Statistical analysis was performed using SPSS software, version 20.0, considering a significance level of 5%.

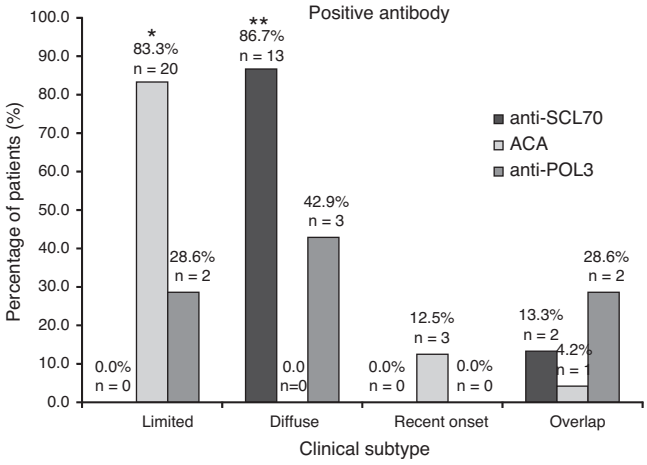

Fig. 1 – Percentage of patients with positive antibody to anti-topo I, ACA and anti-RNAP III among patients with different clinical subtypes of the disease. Each column represents the percent of patients. *Significant difference compared to patients with positive anti-topo I and anti-RNAP III, in limited scleroderma. **Significant difference compared to patients with positive ACA, in diffuse scleroderma (chi-square test; $p < 0.050$).

Results

Of the 46 patients included, 45 were women (97.8%) and 1 was a man (2.2%), with a mean age of 50.21 ± 3.55 years (mean ± standard error).

The race of the patients was as follows: 23 patients were classified as Caucasians (50.0%), 21 as mixed (45.7%) and 2 patients, black (4.3%).

Regarding the diagnosis, 42 diagnosed patients met the 2013 ACR/EULAR classification Criteria for SSc (91.3%). The 4 patients (8.7%) who did not meet these criteria met LeRoy/Medsger's criteria for early SSc.

Regarding the clinical subtypes of the disease, 22 patients had limited cutaneous SSc (47.8%), 16 patients had the diffuse cutaneous SSc (34.8%), 3 patients had the early form (6.5%), 5 patients had overlap form (10.9%) and none had the Sine Scleroderma form. These results are shown in Fig. 1.

Regarding time since diagnosis, 12 patients were diagnosed for more than 10 years (26.1%), 23 patients were diagnosed between 5 and 10 years (50.0%) and 11 patients were diagnosed for less than 5 years (23.9%).

The time for disease progression of patients in general was 9.38 ± 3.08 years.

Among all patients, 24 showed positive ACA (52.2%), 15 were positive for anti-topo I (32.6%) and 7 were positive for anti-RNAP III (15.2%).

Results regarding socio-demographic and clinical parameters in patients with positive ACA, anti-topo I or anti-RNAP III, are presented in Table 1.

There was no significant difference between patients with positive anti-topo I, ACA or anti-RNAP III antibodies in relation to the quantitative variables age, duration of RP before diagnosis, and duration of illness without counting RP. Moreover, SHAQ of patients with positive anti-topo I was significantly higher than that for patients with positive ACA or anti-RNAP III antibody ($p < 0.05$). The same result was observed in relation to the scale of activity. Moreover, SHAQ among patients with positive ACA or anti-topo I was higher than that for patients with positive anti-RNAP III antibody ($p < 0.05$).

For the scale of severity, the score among patients with positive anti-topo I antibody was higher than that for patients with positive ACA antibody ($p < 0.05$), but with no difference for patients with positive anti-RNAP III antibody ($p > 0.05$). There was no further association between positive anti-topo I, ACA or anti-RNAP III antibodies and nominal or ordinal qualitative variables of gender, race, time since diagnosis and 2013 ACR/EULAR classification criteria for SSc. However, there was an association between positive anti-topo I, ACA or anti-RNAP III antibodies and the clinical subtype of the disease ($p < 0.001$), with the percentage of patients with limited disease among patients with positive ACA antibody (83, 3%, $n = 20$) being significantly higher than that among patients with positive anti-topo I and anti-RNAP III antibody (0.0%, $n = 0$ and 28.6%, $n = 2$, respectively). On the other hand, the percentage of patients with diffuse disease, among patients with positive anti-topo I and anti-RNAP III (86.7%, $n = 13$ and 42.9%, $n = 3$), respectively, was significantly higher than that among patients with positive ACA (0.0%, $n = 0$) antibody.

Table 2 shows the results for the skin, vascular and musculoskeletal manifestations in patients with a positive result for anti-topo I, ACA and anti-RNAP III antibodies. In this evaluation, it was observed that the percentage of patients with positive anti-topo I antibody, which had objective RP (93.3%, $n = 14$) was significantly higher than that of patients with positive anti-RNAP III antibody, which also showed objective RP (42.9%, $n = 3$, $p < 0.05$). On the other hand, the percentage of patients with positive anti-RNAP III antibody, who had subjective RP (57.1%, $n = 4$), was significantly higher than that of

Table 1 – Demographic and clinical features of patients with positive anti-topo I, ACA or anti-RNAP III antibodies.

Variable	Positive antibody			*p*-Value
	Anti-topo I	ACA	Anti-RNAP III	
Demographics				
Age	46.27 ± 1.82	54.21 ± 2.72	50.14 ± 6.10	0.149
Sex				
Male	6.7 (1)	0.0 (0)	0.0 (0)	0.348
Female	93.3 (14)	100.0 (24)	100.0 (7)	
Race				
Caucasian	33.3 (5)	54.2 (13)	71.4 (5)	0.516
Mixed	60.0 (9)	41.7 (10)	28.6 (2)	
Black	6.7 (1)	4.2 (1)	0.0 (0)	
Length of diagnosis				
Less than 5 years	13.3 (2)	25.0 (6)	42.9 (3)	0.384
Between 5 and 10 years	53.3 (8)	45.8 (11)	57.1 (4)	
More than 10 years	33.3 (5)	29.2 (7)	0.0 (0)	
Time of RP before diagnosis	2.27 ± 0.68	5.87 ± 1.87	0.86 ± 0.34	0.136
Time of disease without counting RP	7.53 ± 1.19	9.17 ± 5.56	11.43 ± 2.51	0.296
ACR/EULAR criteria				
Yes	100.0 (15)	83.3 (20)	100.0 (7)	0.134
No	0.0 (0)	16.7 (4)	0.0 (0)	
Clinical subtype				
Limited	0.0 (0)b	83.3 (20)[a]	28.6 (2)[b]	<0.001
Diffuse	86.7 (13)[a]	0.0 (0)[b]	42.9 (3)[a]	
Recent onset	0.0 (0)[a]	12.5 (3)[a]	0.0 (0)[a]	
Overlap	13.3 (2)[a]	4.2 (1)[a]	28.6 (2)a	
Sine scleroderma	0.0 (0)[a]	0.0 (0)[a]	0.0 (0)[a]	
Outcome measurements				
SHAQ	0.90 ± 0.11[a]	0.64 ± 0.08[a]	0.16 ± 0.06[b]	<0.001
Severity Scale	6.47 ± 0.80[a]	4.25 ± 0.48[b]	4.43 ± 1.04[ab]	0.044
Activity scale	3.20 ± 0.35[a]	1.94 ± 0.21[b]	1.64 ± 0.37[b]	0.002

The results are presented as mean ± standard error of the mean or relative frequency (absolute frequency).
p-Value in one-way ANOVA or chi-square test.
Different letter on lines indicate significant difference among antibodies.
ACA, anticentromere antibodies; anti-topo I, anti-DNA topoisomerase I antibodies; anti-RNAP III, anti RNA polymerase III antibodies; RP, Raynaud's phenomenon; ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; SHAQ, Scleroderma Health Assessment Questionnaire.

patients with positive anti-topo I antibody, which also showed subjective RP (6.7%, $n = 1$).

Patients with positive anti-topo I antibody showed more digital pitting scars of fingertips than patients with positive ACA antibody ($p < 0.05$). Furthermore, patients with positive ACA antibody had more telangiectasia than patients with positive anti-topo I antibody ($p < 0.050$). Furthermore, patients with positive anti-RNAP III antibody had more muscle atrophy than patients with positive ACA antibody ($p < 0.050$). These results are presented in Fig. 2. For the other variables related to skin, vascular and musculoskeletal manifestations, there was no association between them and the positivity for anti-topo I, ACA and anti-RNAP III antibodies. There was also no significant difference between patients with positivity for these three antibodies in relation to skin score ($p = 0.065$).

The results related to gastrointestinal, cardiopulmonary and renal manifestations in patients with positive anti-topo I, ACA or anti-RNAP III are presented in Table 3. There was no association between the presence of autoantibodies specific for SSc and the variables studied.

The results of the laboratory tests (ESR, CRP, CPK, creatinine, C3 and C4), antibody tests (anti-Ro, anti-La, anti-Sm, anti-RNP and anti Jo-1) and changes observed on hand radiographs in patients with positive anti-topo I, ACA and/or anti-RNAP III antibodies are shown in Table 4, where a lack of statistical significance for all the studied parameters can be seen.

Discussion

In our study, a unique sample was defined that was representative of the Midwest region of Brazil, characterized by a heterogeneous group of patients with various spectrums of disease and different stages of clinical manifestations and disease activity, but that is very similar to other patient populations in the country and even from other locations.[33–38]

This study objective was to investigate the correlation between the profile of specific autoantibodies (ACA, anti-topo I or anti-RNAP III) and clinical and laboratory manifestations in this population of patients with SSc. Similar to

Table 2 – Distribution of patients according to the skin, vascular and musculoskeletal manifestations in patients with positive anti-topo I, ACA or anti-RNAP III antibodies.

Variable	Positive antibody			*p*-Value
	Anti-topo I	ACA	Anti-RNAP III	
Skin manifestations				
Calcinosis				
Yes	20.0 (3)	25.0 (6)	14.3 (1)	0.817
No	80.0 (12)	75.0 (18)	85.7 (6)	
Hands				
No changes	13.3 (2)	20.8 (5)	28.6 (2)	0.685
With changes	86.7 (13)	79.2 (19)	71.4 (5)	
Findings on hands (n = 37)				
Edematous phase	15.4 (2)	36.8 (7)	20.0 (1)	0.735
Indurative phase	46.2 (6)	31.6 (6)	40.0 (2)	
Atrophic phase	38.5 (5)	31.6 (6)	40.0 (2)	
Skin score (Rodnan modified)	16.33 ± 2.03	10.79 ± 1.30	13.86 ± 2.80	0.065
Vascular manifestations				
RP				
Objective	93.3 (14)[a]	66.7 (16)[ab]	42.9 (3)[b]	0.036
Subjective	6.7 (1)[b]	33.3 (8)[ab]	57.1 (4)[a]	
Absent	0.0 (0)	0.0 (0)	0.0 (0)	
Digital pitting scars of fingertips				
Yes	53.3 (8)[a]	16.7 (4)[b]	14.3 (1)[ab]	0.031
No	46.7 (7)[b]	83.3 (20)[a]	85.7 (6)[ab]	
Active ulcers				
Yes	20.0 (3)	8.3 (2)	0.0 (0)	0.316
No	80.0 (12)	91.7 (22)	100.0 (7)	
Necrosis or amputation				
Yes	13.3 (2)	12.5 (3)	0.0 (0)	0.602
No	86.7 (13)	87.5 (21)	100.0 (7)	
Telangiectasias				
Yes	53.3 (8)[b]	87.5 (21)[a]	57.1 (4)[ab]	0.045
No	46.7 (7)[a]	12.5 (3)[b]	42.9 (3)[ab]	
Musculoskeletal manifestations				
Arthritis/synovitis				
Yes	40.0 (6)	37.5 (9)	0.0 (0)	0.134
No	60.0 (9)	62.5 (15)	100.0 (7)	
Flexion contracture				
Yes	26.7 (4)	8.3 (2)	14.3 (1)	0.300
No	73.3 (11)	91.7 (22)	85.7 (6)	
Tendon friction rubs				
Yes	6.7 (1)	4.2 (1)	0.0 (0)	0.773
No	93.3 (14)	95.8 (23)	100.0 (7)	
Muscle weakness				
Yes	20.0 (3)	8.3 (2)	28.6 (2)	0.347
No	80.0 (12)	91.7 (22)	71.4 (5)	
Atrophy				
Yes	6.7 (1)[ab]	4.2 (1)[b]	42.9 (3)[a]	0.012
No	93.3 (14)[ab]	95.8 (23)[a]	57.1 (4)[b]	

The results are presented as mean ± standard error of the mean or relative frequency (absolute frequency).
p-Value in one-way ANOVA or chi-square test.
Different letter on lines indicate significant difference among antibodies.
ACA, anticentromere antibodies; anti-topo I, anti-DNA topoisomerase I antibodies; anti-RNAP III, anti RNA polymerase III antibodies; RP, Raynaud's phenomenon.

Table 3 – Distribution of patients according to gastrointestinal, cardiopulmonary and renal manifestations in patients with positive anti-SCL70, ACA or anti-POL3.

Variable	Positive antibody			*p*-Value
	Anti-topo I	ACA	Anti-RNAP III	
Gastrointestinal manifestations				
Esophagus involvement				
Yes	73.3 (11)	70.8 (17)	57.1 (4)	0.730
No	26.7 (4)	29.2 (7)	42.9 (3)	
Other GI manifestations				
GERD	26.7 (4)	16.7 (4)	0.0 (0)	0.304
Esophagitis	20.0 (3)	16.7 (4)	42.9 (3)	0.329
Gastritis	13.3 (2)	20.8 (5)	14.3 (1)	0.812
Esophagic hypotonia	20.0 (3)	25.0 (6)	0.0 (0)	0.340
Esophageal dilation	6.7 (1)	4.2 (1)	14.3 (1)	0.634
Cardiopulmonary manifestations				
VFC	76.27 ± 3.27	86.50 ± 2.56	83.71 ± 4.47	0.054
VFC – classification				
>80%	40.0 (6)	70.8 (17)	42.9 (3)	0.282
Between 70 and 80%	40.0 (6)	20.8 (5)	57.1 (4)	
Between 50 and 69%	13.3 (2)	8.3 (2)	0.0 (0)	
<50%	6.7 (1)	0.0 (0)	0.0 (0)	
Chest CT				
Normal	26.7 (4)	62.5 (15)	42.9 (3)	0.089
Altered	73.3 (11)	37.5 (9)	57.1 (4)	
Findings on CT (n = 24)				
Fibrosis	72.7 (8)	66.7 (6)	75.0 (3)	0.938
"Ground-glass" pattern	27.3 (3)	33.3 (3)	25.0 (1)	
Echo PSAP	32.50 ± 5.50	37.25 ± 3.64	32.67 ± 12.25	0.882
Echocardiogram (n = 26)				
Normal	40.0 (6)	54.2 (13)	14.3 (1)	0.164
Altered	60.0 (9)	45.8 (11)	85.7 (6)	
Findings on echocardiogram				
Valvulopathy	33.3 (5)	25.0 (6)	28.6 (2)	0.854
Concentric LVH	20.0 (3)	12.5 (3)	28.6 (2)	0.583
LV diastolic dysfunction	6.7 (1)	16.7 (4)	28.6 (2)	0.395
Mild or moderate PAH	6.7 (1)	8.3 (2)	28.6 (2)	0.260
Pericarditis	13.3 (2)	12.5 (3)	0.0 (0)	0.602
Renal manifestations				
Renal crisis				
Yes	0.0 (0)	0.0 (0)	0.0 (0)	–
No	100.0 (15)	100.0 (24)	100.0 (7)	

The results are presented as mean ± standard error of the mean or relative frequency (absolute frequency).
p-Value in one-way ANOVA or chi-square test.
ACA, anticentromere antibodies; anti-topo I, anti-DNA topoisomerase I antibodies; anti-RNAP III, anti-RNA polymerase III antibodies; GI, gastrointestinal; GERD, gastroesophageal reflux disease; VFC, vital functional capacity; CT, computed tomography; Echo PSAP, pulmonary artery estimated pressure by transthoracic echocardiogram; LVH, left ventricle hypertrophy; LV, left ventricle; PAH, pulmonary artery hypertension.

other populations, our patients were mostly female (97.83%) and with limited scleroderma (47.8%), with a mean age of 50 years and caucasian (50.0%). In 65% of the patients, RP was the first disease manifestation before diagnosis, time since diagnosis occurred mainly between 5 and 10 years (50.0%), mean disease duration of 9 years, and average modified Rodnan skin score of 13.66. Regarding specific antibodies, 52.2% of the patients had positive ACA, 32.6% were positive for anti-topo I and 15.2% were positive for anti-RNAP III.

In Brazil, recently two different groups of researchers in the southern region described the occurrence of major specific autoantibodies in patients with SSc and both authors highlighted the importance of autoantibodies in the evaluation of patients with SSc.[33,34]

The first group from Hospital Evangélico de Curitiba (HUEC) found, in 66 SSc patients, the prevalence of ACA, anti-topo I and anti-U1-RNP, respectively in 33.3%, 17.8% and 11.8% of patients.[33] Although the percentage of each autoantibody found in our study is greater for ACA and anti-topo I, the order of distribution among specific autoantibodies was identical. The authors from HUEC also observed an association of anti-topo I with diffuse scleroderma and digital pitting scars of

Table 4 – Distribution of patients according to the laboratory tests in patients with anti-topo I, ACA or anti-RNAP III positive.

Variable	Positive antibody			*p*-Value
	Anti-topo I	ACA	Anti-RNAP III	
ESR	37.00 ± 6.23	22.63 ± 3.58	22.86 ± 5.06	0.076
CRP	13.82 ± 6.56	9.53 ± 2.72	7.36 ± 2.29	0.664
CPK	136.00 ± 45.85	120.83 ± 13.48	107.43 ± 15.68	0.846
C3	122.33 ± 5.69	138.00 ± 6.49	124.29 ± 9.16	0.199
C4	28.73 ± 2.21	35.38 ± 2.25	36.14 ± 1.92	0.089
Anti-Ro				
Positive	26.7 (4)	4.2 (1)	0.0 (0)	0.054
Negative	73.3 (11)	95.8 (23)	100.0 (7)	
Anti-La				
Positive	6.7 (1)	0.0 (0)	0.0 (0)	0.348
Negative	93.3 (14)	100.0 (24)	100.0 (7)	
Anti-RNP				
Positive	26.7 (4)	4.2 (1)	28.6 (2)	0.092
Negative	73.3 (11)	95.8 (23)	71.4 (5)	
Anti-Jo 1				
Positive	6.7 (1)	4.2 (1)	0.0 (0)	0.773
Negative	93.3 (14)	95.8 (23)	100.0 (7)	
Hand X-ray				
Normal	53.3 (8)	50.0 (12)	71.4 (5)	0.603
Altered	46.7 (7)	50.0 (12)	28.6 (2)	
Findings on hand X-ray (n = 21)				
Calcinosis	14.3 (1)	50.0 (6)	50.0 (1)	0.283
Reabsorption (proximal phalange)	85.7 (6)	50.0 (6)	50.0 (1)	

The results are presented as mean ± standard error of the mean or relative frequency (absolute frequency).
p-Value in one-way ANOVA or chi-square test.
ACA, anticentromere antibodies; anti-topo I, anti-DNA topoisomerase I antibodies; anti-RNAP III, anti RNA polymerase III antibodies; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CPK, creatine phosphokinase; C3, C3 complement component; C4, C4 complement component.

fingertips; however, they found an association between this autoantibody and the presence of cardiomyopathy. Unlike our study, ACA was protective for cardiomyopathies and anti-U1-RNP was more common in overlap forms.[33]

The second group from Hospital de Clínicas of the Federal University of the state of Paraná (HC-UFPR) investigated the prevalence of anti-RNAP III, anti-topo I and ACA in 85 SSc patients and found their presence in 41.18%; 31.76% and 30.59% of patients, respectively.[34] Although it was noted that the limited form was the most prevalent among patients, this study found very high prevalence of positive anti-RNAP III, which is related to diffuse cutaneous SSc. Our study has validated the same clinical features observed in the group of patients from HC-UFPR who were anti-topo I-positive, such as association with diffuse scleroderma, the presence of active disease and digital ulcers. However, the group from HC-UFPR found an association between synovitis and positivity for anti-RNAP III, and greater prevalence of systemic hypertension and cardiac conduction block in patients with positive ACA.[34]

In the present work, ACA was mainly correlated with limited cutaneous SSc, with earlier onset of disease, as well as higher prevalence of telangiectasias. ACA was found in 52.2% of patients and, in the literature, ACAs were observed in about 20–30% of patients with SSc[10,12] and in 55–80% of patients with the limited form[9], although it can vary among different ethnic populations.[10,12] ACAs have predictive value for future development of SSc in patients with RP and are associated with limited cutaneous involvement, peripheral vascular damage and calcinosis.[10,12] However, no greater prevalence of calcinosis in our patients with the limited form was observed. The presence of ACA generally provides a better prognosis than that seen with other antibodies, since they are less frequently associated with interstitial lung fibrosis,[10,12] as observed in our study, although not achieving statistical significance.

In this study, the anti-topo I autoantibody was mainly correlated with the diffuse cutaneous SSc, with greater disease severity and activity, with worse quality of life as measured by SHAQ index, a higher prevalence of objective RP, and digital pitting scars of fingertips. We found anti-topo I in 32.6% of patients, in accordance with the literature, which describes this antibody in 40% of patients with SSc,[12] in about 28–70% patients with the diffuse cutaneous SSc[9,10,12] and in less than 10% of patients with limited scleroderma.[9–11] As observed in our patients, it is described that ethnic differences significantly affect the prevalence of anti-topo I, and it is observed to a lesser extent in Caucasians. Anti-topo I, when determined by immunodiffusion, is virtually never seen in healthy individuals, in other diseases of the connective tissue, or in patients with primary RP.[10,12] Its presence is described as related to worse prognosis, global disease activity,

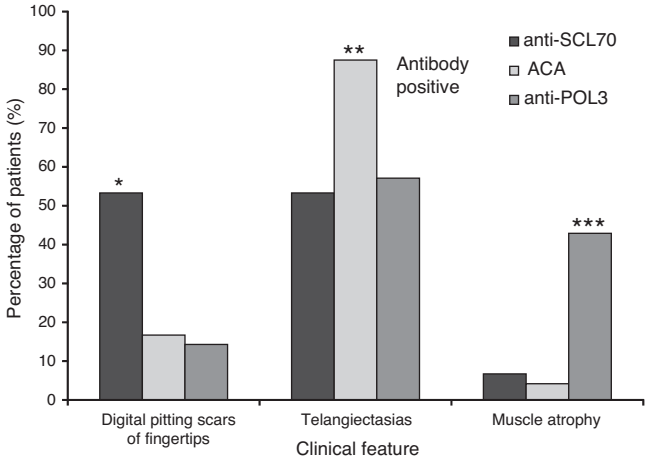

Fig. 2 – Percentage of patients with clinical manifestations such as digital pitting scars of fingertips, telangiectasias and muscle atrophy, among patients with positive anti-topo I, ACA and anti-RNAP III. Each column represents the percent of patients. *Significant difference compared to patients with positive ACA and anti-RNAP III. **Significant difference compared to patients with positive anti-topo I and anti-RNAP III. ***Significant difference compared to patients with positive anti-topo I and ACA (chi-square test; $p < 0.050$).

severity of skin involvement, interstitial lung disease and cardiac involvement.[9,10,12,39–41] However, cardiopulmonary manifestations were not more prevalent or more severe in this study.

However, in this work, the anti-RNAP III was primarily correlated with diffuse scleroderma, since other two patients with inflammatory myopathy-associated positive anti-RNAP III had diffuse cutaneous involvement. Interestingly, both patients with overlap were not positive for anti-Jo1 antibody. Moreover, we observed a higher frequency of subjective RP and muscle atrophy in our patients. We found anti-RNAP III in 15.2% of patients, but other published studies describes a prevalence of this antibody at different frequencies, probably related to genetic and racial differences. Its prevalence ranged from 4 to 9.4% in French, 12% in English, 6% in Japanese, 19.4% in Canadian, and 25% in American patients.[9,42] Autoantibodies against RNA polymerase 1 and 3 usually coexist in a prevalence of 20%, and this pattern is highly specific for SSc.[9,10,12] Anti-RNAP III also has a prognostic role, since it was related to diffuse skin involvement, tendon friction rubs, and kidney involvement,[9,42,43] but no scleroderma renal crisis was observed in our patients. Besides myositis observed in our patients, studies published in the literature highlights other significant associations between the positivity of anti-RNAP III with the occurrence of synovitis and systemic hypertension, as well as a possible relation to malignancies, predominantly solid organ cancer.[42,43]

Unlike systemic lupus erythematosus (SLE), the production of a specific autoantibody is unique in patients with SSc, so the occurrence of more than one type of antibody in a patient is rare, except for antibodies against RNA polymerase.[9,10,12] The coexistence of anti-topo I and ACA in SSc is uncommon (0.5–5.5%), although some authors have previously considered it mutually exclusive.[44] Although the correlation between antibodies that define subtypes of SSc is unusual, the coexistence of ACA or anti-topo I with anti-histone antibodies, ACA with anti-mitochondrial antibodies, anti-topo I with anticardiolipin antibodies, ACA or anti-topo I with Ro (SSA) antibodies, or anti RNPs with anti Th/To antibodies was described.[45] Our study observed the coexistence of positive anti-topo I and anti-RNAP III in a patient with diffuse scleroderma.

Based on SHAQ, this study observed higher scores of disability among patients with positivity of anti-topo I, similar to those described in other populations of patients with the diffuse form of SSc.[29,46] Comparatively, Morita and colleagues reported that patients with the diffuse form of SSc had higher rates of disability on the SHAQ, also higher than those of patients with RA, SLE and other autoimmune diseases.[46] It was also observed that SSc patients with articular involvement had higher scores on SHAQ than patients with psoriatic arthritis, while the pain domain was higher in SSc patients than in RA patients.[47] Unpublished data of our patients confirm the greater disability in the subgroup of patients with arthritis. Recently Iudici and colleagues found that patients with early form of SSc, despite having only RP, had already experienced an impairment of quality of life in both physical and mental domains.[48] Accordingly, our 3 patients with the early form showed a disability index measured by the SHAQ that was comparable to other groups. The usefulness of SHAQ in the evaluation of patients with SSc has been demonstrated by studies that reported that it can predict the evolution and survival in these patients.[29,49] In this work, there was a significant positive linear correlation between SHAQ and disease activity as measured by the Pearson test. Medsger and colleagues found that the rates of disability measured by SHAQ showed strong correlation with skin thickening, cardiac involvement, digital contractures, tendon friction rubs, and renal involvement in 1000 patients with SSc.[26]

In conclusion, this study confirms the important role of specific autoantibodies in the evaluation of patients with SSc. It is possible to correlate the antibody profile of this national population with some distinct clinical manifestations of the disease.

Conclusions

We highlight, in agreement with the literature, that the clinical subtype of the disease and some clinical manifestations in SSc may correlate positively with the presence of specific autoantibodies.

The presence of ACA was observed, particularly in the early forms of the disease, limited scleroderma, and overlap syndrome, with absence in the diffuse scleroderma. The anti-topo I was mainly observed in the diffuse and overlap forms, being absent in the limited disease.

Patients with positive anti-topo I have higher disease activity and severity, and impairment in quality of life as measured by SHAQ index.

Anti-topo I-positive patients have more objective RP and digital pitting scars of fingertips, patients positive for ACA had

more telangiectasia and patients with positive anti-RNAP III antibody had more muscle atrophy.

The specific autoantibodies may directly contribute to the patient's evolution and prognosis.

Conflicts of interest

The authors declare no conflicts of interest.

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