Autoantibody profile and clinical correlation in a group of patients with systemic sclerosis in southern Brazil

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ABSTRACT

Objectives: To assess the manifestations of systemic sclerosis (SSc), with an emphasis on the analysis of autoantibodies and their clinical correlations, in a population of patients followed up at the SSc Outpatient Clinics of the Hospital de Clínicas of the Universidade Federal do Paraná. **Methodology:** Cross-sectional study with 96 patients followed up at the SSc Outpatient Clinics of the hospital between September 2007 and September 2009. **Results:** Most patients were of the female sex, in their forties or fifties, and the median time of disease was ten years. The limited cutaneous form of SSc was more prevalent. The analysis of the autoantibodies showed the association of anticentromere antibody (ACA) with the following: the limited form of SSc; more advanced age at the time of diagnosis; longer disease time; longer interval between the appearance of the Raynaud's phenomenon (RyP) and the first non-RyP symptom; systemic arterial hypertension (SAH); and cardiac conduction blocks. The antitopoisomerase-1 antibody (ATA-1, previously called anti-Scl-70) was more common in the presence of the diffuse form of SSc, active disease, and digital ulcers. The anti-RNA polymerase III antibody (anti-Pol III) correlated with the diffuse form of SSc, disease activity, and synovitis. **Conclusions:** This study emphasizes and confirms the important role of autoantibodies in assessing patients with SSc, allowing the correlation between the autoimmune profile of patients with SSc and specific manifestations of the disease.

Keywords: systemic sclerosis; autoimmunity; autoantibodies.

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INTRODUCTION

Systemic sclerosis (SSc), also known as scleroderma (*skleros*: hard; *derma*: skin), is a chronic disease of still unknown etiology that affects multiple organ systems. It is characterized by structural and functional abnormalities of small blood vessels, fibrosis of the skin and internal organs, and immune dysregulation. Based on the extension of cutaneous involvement, SSc is classified into two major subtypes: the limited and diffuse forms.[1] Autoantibodies are commonly present in both the diffuse and limited forms of SSc. The role played by autoantibodies in the pathogenesis of SSc has been discussed, although correlations between the autoantibodies and the clinical manifestations of SSc have been well established.[2] In SSc, the antinuclear antibody (ANA) is present in more than 95% of the patients,[3,4] independently of the clinical form of the disease. The target of the anticentromere antibody (ACA) is the centromere proteins of the cell nucleus. That antibody is

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associated with the limited form of SSc and has a protective role in the disease, because it is associated with a lower incidence of pulmonary fibrosis.[4] The anti-topoisomerase-1 antibody (ATA-1), present in up to 20%-30% of scleroderma patients, is associated with the diffuse form of SSc, indicating more severe disease, of worse prognosis and higher mortality.[4] The anti-RNA polymerase III antibody (anti-Pol III) is also associated with a worse prognosis, the diffuse form of SSc, scleroderma renal crisis, and higher mortality.[5] The SSc Outpatient Clinics of the Hospital de Clínicas of the UFPR provides care for more than 120 scleroderma patients. This study aimed at identifying, recording and analyzing that population of patients, describing their demographic, clinical and laboratory aspects, with an emphasis on their immune profile.

PATIENTS AND METHODS

The study comprised SSc patients followed up at the Service of Rheumatology of the Hospital de Clínicas of the UFPR. The study inclusion period was from September 2007 to September 2009. A written informed consent approved by the Committee of Ethics in Research with Human Beings of the hospital was obtained from all patients by their physicians prior to study inclusion. The patients' admission in the study met the following inclusion criteria: a) follow-up at the SSc Outpatient Clinics of the Service of Rheumatology of the Hospital de Clínicas of the UFPR; b) diagnosis of SSc according to the classification criteria defined by the American College of Rheumatology (ACR);[6] and c) age over 16 years. Patients with the following characteristics were ineligible for the study: a) scleroderma-like diseases; b) localized scleroderma; c) mixed connective tissue disease; and d) refusal to sign the written informed consent. Initially, 108 patients were assessed to enter the study, 12 of whom were excluded as follows: one with localized scleroderma; two diagnosed with stiff skin syndrome; and nine who did not meet the ACR criteria for the classification of SSc. The remaining 96 patients were included in the study. No patient was excluded after being included in the study.

Data collection

The collection of demographic and clinical data, complementary exam findings, and antibody profile followed the routine of the SSc Outpatient Clinic of the Hospital de Clínicas of the UFPR. The complementary exams, except for the measurement of autoantibodies, were all performed at the hospital according to the reference values of each service. The analysis of the ANA, ACA, ATA-1, and anti-Pol III autoantibodies was performed at the Laboratório Metanalysis - Centro de Diagnósticos Médicos, in the city of Porto Alegre, state of Rio Grande do Sul, and funded by the Service of Rheumatology of the Hospital de Clínicas of the UFPR.

Analysis of autoantibodies

The analysis of the ANA and ACA autoantibodies by use of indirect immunofluorescence (IIF) with HEp-2 cells (human larynx epidermoid carcinoma cell line) was screened at 1:80.[7] For the analysis of the ATA-1 autoantibodies, the kit QUANTA LiteTM Scl-70 of the INOVA Laboratory (INOVA Diagnostics, Inc; San Diego, CA, USA) was used. According to the kit manufacturer, the sample was classified as follows: non-reactive if < 20 units; poorly reactive, between 20 and 39 units; moderately reactive, between 40 and 80 units; and strongly reactive (high values) if > 80 units. The anti-Pol III autoantibody measurement was performed by use of ELISA (Enzyme Linked Immunoassay) for the semiquantitative detection of the anti-Pol III IgG antibodies in the serum of patients. The kits were provided by the INOVA and MBL (Medical and Biological Laboratories Co. Ltd., Nagoya, Japan) laboratories. According to the INOVA kit, the sample was classified as follows: non-reactive if < 20 units; poorly reactive, between 20 and 39 units; moderately reactive, between 40 and 80 units; and strongly reactive (high values) if > 80 units. According to the MBL kit, values ≥ 28 U/mL were considered positive.

Statistical analysis

The statistical analysis was performed by using the JMP 7.0® statistical software (SAS Institute, Inc., Cary, NC, USA). The statistical significance level adopted was 5% ($P < 0.05\%$). Data with normal distribution were presented as mean ± standard deviation, and non-normal data were presented as mean and 25% and 75% interquartile values. Normal data were compared by using the χ^2 test or Student *t* test for two variables, and ANOVA for more than two variables. Likewise, non-normal data were compared by using the Wilcoxon test for two variables, and the Kruskal-Wallis test for more than two variables. The "n" value (number of the sample) of the data assessed was only specified when it did not correspond to the totality of patients.

RESULTS

Demographic data

The mean age of the 96 patients was 49.27 ± 12.55 years, and 88 (91.67%) were of the female sex. The mean body mass index (BMI) (n = 71) was 23.69 ± 3.63. The ethnicities of the patients (n = 92) were as follows: 72 Caucasian (78.26%);

four black (4.35%); and 16 mixed (17.39%). Of all patients included in the study, 63 (65.62%) had the limited cutaneous form of SSc, 25 (26.04%) had the diffuse cutaneous form, and eight (8.33%) were classified as "other forms" [overlap of SSc and rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), polymyositis (PM) or juvenile idiopathic arthritis (JIA)]. The estimated median time of disease was ten years (5.25 and 21.00, interquartiles).

Autoantibodies

The serological sample of 85 patients was available for analysis of autoantibodies. The ANA was positive in most patients

Table 1
Correlation between the autoantibodies studied and the patients' clinical data (anamnesis)

Anamnesis data	Reactive ACA	Reactive ATA-1	Reactive anti-Pol III
N¥	26 (30.59)	27 (31.76)	35 (41.18)
Age*+	54.58 ± 10.53 (P < 0.01)	45.78 ± 12.51	47.2 ± 12.83
Age RyP*+	32.04 ± 16.42	37.70 ± 13.73	33.8 ± 13.95
Age non-RyP*+	41.23 ± 13.87	39.93 ± 12.55	38.43 ± 12.36
Disease time*§	23.00 (9.75 e 30.75) (P < 0.01)	7.00 (4.00 e 10.00) (P < 0.01)	10.00 (6.00 e 22.00)
RyP–non-RyP Interval*§	3.50 (0.75 e 18.50) (P < 0.01)	1.00 (0.00 e 2.00) (P < 0.01)	2.00 (0.00 e 8.00)
Esophageal complaints¥	61.54	66.67	65.71
Gastric complaints¥	30.77	33.33	45.71
Intestinal complaints¥	26.92	14.81(P = 0.05)	25.71
Dyspnea¥	23.08	18.52	22.86
Palpitations¥	34.61 (P = 0.07)	14.81	20.00

§ mean (25% and 75% interquartiles); + (mean ± SD); ¥ (% positivity); * years; P: statistical significance level in the comparison between ACA, ATA-1, and anti-Pol III autoantibodies; RyP: Raynaud's phenomenon.

Table 2
Correlation between the autoantibodies studied and the patients' clinical data (clinical exam)

Clinical exam data	Reactive ACA	Reactive ATA-1	Reactive anti-Pol III
N¥	26 (30.59)	27 (31.76)	35 (41.18)
Rodnan skin score*+	11.50 (7.75 e 20.25)	15.00 (9.00 e 23.00)	16.00 (8.00 e 22.00)
Limited cutaneous form ¥	43.64 (P < 0.01)	21.82 (P < 0.01)	34.54
Diffuse cutaneous form ¥	4.35 (P < 0.01)	52.17 (P = 0.03)	56.52 (P = 0.04)
"Other forms" ¥	14.29	42.86	42.86
Active disease ¥	19.00 (n = 23)	50.00 (n = 24) (P < 0.01)	40.00 (P = 0.05)
RyP ¥	100	96.30	94.29
Digital ulcers ¥	42.31	70.37 (P = 0.01)	60.00
Synovitis ¥	11.54	7.41	20.00 (P = 0.05)
Joint contractions ¥	30.77	25.93	40.00
Tendon friction rub ¥	0	3.70	2.86
Muscle weakness ¥	26.92	29.63	37.14
Muscle atrophy ¥	7.69	14.81	14.29
SAH ¥	53.85 (P < 0.01)	14.81 (P = 0.04)	34.29
Renal crisis ¥	0.00	0.00	0.00

§ mean (25% and 75% interquartiles); ¥ (% positivity); P: statistical significance level in the comparison between ACA, ATA-1, and anti-Pol III autoantibodies; RyP: Raynaud's phenomenon; SAH: systemic arterial hypertension.

(92.94%), followed by anti-Pol III (41.18%), ATA-1 (31.76%), and ACA (30.59%). Except for the anti-Pol III antibody, of which most values were low to intermediate, the majority of the other autoantibodies showed high values. When analyzing the patients' autoimmune profile, significant associations between the different autoantibodies and the results of the clinical and complementary exams were sought. In regard to ACA, the association was positive with the following: the limited form of SSc; more advanced age at the time of diagnosis; longer disease time; longer interval between the appearance of the Raynaud's phenomenon (RyP) and the first non-RyP symptom; systemic arterial hypertension (SAH); and cardiac conduction blocks. The association was negative with pulmonary fibrosis and restrictive disorder on pulmonary function test. The presence of ATA-1 related with the following: the diffuse form of SSc; disease activity (according to the European Scleroderma Study Group criteria);[8] and digital ulcers. Negative association was observed with intestinal complaints and SAH. Regarding anti-Pol III, a positive association was observed with the following: the diffuse form of SSc; disease activity; and synovitis. No negative association of that autoantibody with the parameters assessed was observed. No patient was concomitantly positive for ATA-1 and ACA autoantibodies. However, of the 35 anti-Pol III-positive patients, 11 were concomitantly reactive to ACA and 13 to ATA-1. The correlation of the ACA, ATA-1 and anti-Pol III autoantibodies with the clinical data (anamnesis and clinical exam) and complementary exams is shown in Tables 1, 2, and 3, respectively.

DISCUSSION

This study identified, recorded and analyzed the demographic, clinical and laboratory data, with emphasis on the immune profile, of 96 patients undergoing follow-up at the SSc Outpatient Clinics of the Hospital de Clínicas of the UFPR. Most of the patients studied were in their forties and fifties, in accordance with the age group with the highest prevalence of SSc.[4,9,10] To estimate the age of SSc onset, the disease was considered to have started with the appearance of the RyP, as described in the EUSTAR - Eular Scleroderma Trials and Research.[4] Other studies, however, have considered that the onset of SSc corresponds to the appearance of the first non-RyP symptom.[11,12] Being the disease onset the appearance of either the first RyP symptom or the first non-RyP symptom, in this study, the estimated mean age of SSc onset differed between neither the clinical forms of the disease nor the genders (data not shown). Because SSc is more prevalent in the female gender, the small number of male patients in this study did not surprise. The female:male ratio was 10.2:1, comparable to those reported in other studies, such as in the United Kingdom (3:1)[13] and Japan (14:1),[14] and closer to that reported in Iceland (8:1).[15] Most patients included in this study had the limited form of SSc, reported as the most common clinical form of SSc.[4,16] The serological sample of part of the patients was available for the analysis of autoantibodies. In the patients assessed, prevalence of the ANA, ACA and ATA-1 autoantibodies was found, similarly to that reported in the

Table 3
Correlation between the autoantibodies studied and the data of the complementary exams

Data of Complementary Exams	Reactive ACA	Reactive ATA-1	Reactive anti-Pol III
N¥	26 (30.59)	27 (31.76)	35 (41.18)
Acute-phase proteins¥	30.77	42.31 (n = 26)	37.14
Proteinuria¥	3.85	7.69 (n = 26)	5.71
Elevation in creatine phosphokinase¥	4.17 (n = 24)	4.00 (n = 25)	21.21 (n = 33)
Cardiac conduction block¥	24.00 (n = 25) (P = 0.05)	4.17 (n = 24)	9.09 (n = 33)
CO diffusion capacity +	67.09 ± 23.06	64.92 ± 20.39	69.62 ± 24.23
Reduction in the LV ejection fraction¥	8.33 (n = 24)	4.00 (n = 25)	12.12 (n = 33)
Diastolic dysfunction¥	37.50 (n = 24)	28.00 (n = 25)	33.33 (n = 33)
Fibrosis on chest X-ray¥	12.00 (n = 25) (P < 0.01)	44.00 (n = 25)	29.41 (n = 34)
Pulmonary restrictive disorder¥	16.67 (n = 24) (P = 0.04)	36.00 (n = 25)	33.33 (n = 33)
Pulmonary hypertension¥	33.33 (n = 24)	20.00 (n = 25)	30.30 (n = 33)
Active disease¥	19.00 (n = 23)	50.00 (n = 24) (P < 0.01)	40.00 (P = 0.05)

+ (mean ± SD); ¥ (% positivity); P: statistical significance level in the comparison between ACA, ATA-1, and anti-Pol III autoantibodies; LV: left ventricular.

literature.[1,3,4,17] It was worth noting the high levels of those autoantibodies, as well as the exceptionally high prevalence of anti-Pol III-positive patients (41.18%). When compared to the findings of other studies, the prevalence of the anti-Pol III antibody in our population is, at least, two-fold higher.[17,18] A study from 2007 emphasized the high prevalence of anti-Pol III in North-American patients (25% in that population) as compared with French patients.[19] Studies showing an association between the levels of autoantibodies, especially ATA-1, and clinical manifestations of SSc reinforce the concept that the autoantibodies are not only an epiphenomenon in SSc, but play a role in its pathogenesis.[20-22] In EUSTAR, the status of autoantibodies has contributed more to systemic complications than the clinical subtype of SSc has.[4] In the literature, high levels of ATA-1 have been related to disease activity and greater severity; therefore, we considered that the high levels of ATA-1 and of the other autoantibodies in this study also reflect a more severely affected population. The assessment of patients from a tertiary center might have contributed to the selection of more severe cases. Because this is a cross-sectional study, the prospective analysis to confirm that hypothesis has not been performed.

In this study, similarly to the findings of other studies, the ACA antibody was associated with indicators of better prognosis, differently from the ATA-1 antibody, associated with diffuse SSc and higher severity.[2,3,23] A protective effect of ACA was observed regarding pulmonary fibrosis and restrictive disorder on spirometry; on the other hand, the ATA-1 antibody was associated with a higher prevalence of digital ulcers and active disease, as previously reported by other authors.[4] However, we observed no correlation of the ACA antibody with the presence of pulmonary hypertension (PH), which would be expected considering the higher prevalence of vasculopathy in ACA-positive patients.[3,18] We believe that is because we have not assessed, due to the small "n" of the sample, PH according to the presence of pulmonary fibrosis on simple chest radiography. Walker *et al.*[4] have only reported a correlation between ACA and PH in the absence of pulmonary fibrosis; in the presence of fibrosis, the correlation was positive with ATA-1.

The anti-Pol III antibody, described in SSc only in a few studies,[24-26] has also been associated with indicators of more severe disease. Because of the lack of patients with a history of renal crisis in this study, the association of anti-Pol III and sclerodermic renal crisis could not be assessed. Similarly, the relation between anti-Pol III and mortality in SSc could not be assessed, because this is a cross-sectional study.

Although some studies have shown a possible correlation between the serum levels of autoantibodies and disease activity,[21] Reveille and Solomon[5] have reported that serial measurements of antibodies in patients with positivity once demonstrated are not justified.

CONCLUSIONS

The present study could establish correlations between the autoimmune profile of patients with SSc and specific manifestations of the disease, confirming and emphasizing the importance of assessing autoantibodies in that population.

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