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Presence of autoantibodies and epidemiological characteristics of systemic sclerosis patients in western Paraná

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ABSTRACT. Systemic sclerosis (SSc) is a systemic autoimmune disease of the connective tissue with unknown etiology, characterized by abnormal collagen deposition in the skin. The changes in patients result in the obliteration of microvessels and fibrosis, leading to damage to the skin, gastrointestinal tract, kidneys, lungs, heart, and oral cavity. Brazil has insufficient data about the epidemiological and laboratory aspects of SSc. This study aimed to identify and characterize the patients in treatment for SSc in Cascavel/PR, a city in the south of Brazil. Data were collected from March 2019 to September 2020 from all health services of Cascavel that provide care in rheumatology: a university-affiliated hospital, two public outpatient clinics, and six private clinics. Data about age, sex, residence, time to diagnosis, the form of the disease, and the results of tests for antinuclear (ANA) and anti-extractable nuclear antigens (ENA) antibodies were obtained. The study identified 57 patients; 82.5% were female with a mean age of 50.4 years, and the predominant disease form was diffuse (52.6%). The ANA test was positive in 87.7% of the patients, of which 49.2% showed high titers and a predominance of the centromeric pattern. Anti-Scl-70, anti-centromere (ACA), and anti-RNA polymerase III antibodies were positive in 28.1%, 25%, and 5% of the patients. The characteristics of the patients diagnosed with SSc in Cascavel/PR are similar to those reported in the literature. This study contributed to the scarce Brazilian data about the disease.

Keywords: scleroderma; epidemiology; Brazil; antinuclear antibodies; anti-ENA antibodies.

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Introduction

Systemic sclerosis (SSc) is a chronic inflammatory disease of the connective tissue, with a complex etiopathogenesis characterized by autoantibodies and varying degrees of tissue fibrosis and small vessel vasculopathy (LeRoy & Medsger Jr, 2001). Although its etiopathogenesis is not fully understood, the pathophysiology has three main strands: fibroblast hyperactivity, proliferative microangiopathy, and immune disorders suggestive of autoimmunity (Pappas-Taffer, 2018). Several organs can be affected, especially the skin, lung, heart, kidneys, and gastrointestinal tract, with SSc having a heterogeneous phenotypic expression and the predominant visceral involvement determining the disease prognosis (Steen & Medsger Jr, 2000; Muangchan et al., 2013).

The classification criteria proposed in 1980 by the American College of Rheumatology (ACR) efficiently identifies patients with a well-defined disease, and the condition was later classified into limited and diffuse clinical forms. In the 21st century, with the new criteria proposition for SSc sine scleroderma, early SSc, and very early SSc, the spectrum of the disease was considerably increased, also allowing the early diagnosis of a significant number of patients (Masi et al., 1980; Leroy et al., 1988). Incorporating nailfold capillaroscopy (NFC) and specific autoantibody data allowsfor a more sensitive classification system for detecting early disease (Leroy & Medsger Jr, 2001). Thus, the European Alliance of Associations for Rheumatology (EULAR) and the European Scleroderma Trials and Research group (EUSTAR) published the new classification criteria for SSc in 2013, which are more sensitive and specific than the 1980 ACR criteria and have been used since (Van Den Hoogen et al., 2013).

The extent of cutaneous involvement categorizes the disease into the following subtypes: diffuse cutaneous form (skin thickening proximal to the elbows and knees); limited cutaneous form (skin thickening distal to the elbows and knees, which may also affect the face), and 'sine scleroderma' form (exclusive visceral involvement, without evidence of cutaneous involvement) (Claire & Cacoub, 2016).

More than 80% of SSc patients have autoantibodies. Some of these antibodies have a restricted association with SSc, giving them considerable diagnostic value. The autoimmune response in SSc is more limited than in other autoimmune diseases, with the serum of an individual patient usually containing only a single autoantibody type (Stochmal, Czuwara, Trojanowska, & Rudnicka, 2020). The diffuse cutaneous form has traditionally been associated with a more aggressive course, with early involvement of internal organs and the presence of the anti-topoisomerase I antibody (anti-Scl-70). The limited cutaneous form, in general, is associated with a slower evolution, often associated with pulmonary arterial hypertension (PAH) and the presence of the anticentromere antibody (ACA) (Leroy et al., 1988; Stochmal et al., 2020).

Brazil's literature about autoimmune diseases is scarce compared to developed countries (Nikpour, Stevens, Herrick, & Proudman, 2010; Barnes & Mayes, 2012), necessitating epidemiological, clinical, and laboratory data from other localities in Brazilian manuals and guides. Therefore, this study aimed to detect the frequency of autoantibodies found in patients diagnosed with SSc in the health services of Cascavel, Paraná, adding data to the national literature and providing local data about SSc to support Brazilian health policies.

Material and methods

Patients

A cross-sectional and descriptive study was carried out by reviewing the medical records of patients diagnosed with SSc in Cascavel/PR in all clinics that practice rheumatology [six private rheumatology clinics in a regional public center of specialties (Regional Center of Specialties of the Intermunicipal Health Consortium of Western Paraná CRE/CISOP), at the municipal specialty center (Center for Specialized Care) and the specialty outpatient clinic of the Western Paraná University Hospital (HUOP)]. The medical records were accessed in the clinics, and data were collected using a worksheet specially developed for this purpose. The data collection period was between March 2019 and September 2020.

Epidemiological (age, sex, place of residence, and time to diagnosis), clinical (diffuse or limited form), and autoantibody [antinuclear antibodies (ANA) and autoantibodies against extractable nuclear antigens (ENA) tests] data were collected.

Patients of both sexes and any age were eligible if they had a diagnosis of SSc with follow-up at health services in Cascavel/PR. They were residents of the host city (Cascavel) or the other 24 municipalities belonging to the 10th Paraná Health Region, with around 500,000 inhabitants.

The Western Paraná State University (UNIOESTE) Research Ethics Committee approved the project (number 2.443.066 and CAAE 79791617.7.0000.0107).

Statistical analysis

Statistical analyses were performed using the chi-square test ($p < 0.05$) in Microsoft Office Excel version 345 software with the XLSTAT package. Data are presented as frequency (%) in contingency tables.

Results

Fifty-seven patients diagnosed with SSc were identified in public and private clinics in Cascavel/PR during the period covered. Of these, 47 (82.5%) were female, with a mean age of 50.4 years (min. 28, max. 81, SD ±11.1). Thirty (52.6%) were residents in Cascavel, with 30 (52.6%) presenting the diffuse form of the disease. The other clinical-epidemiological characteristics are shown in Table 1. The female-to-male ratio was 4.7, with a significant difference between the sexes ($p < 0.0001$). The Chi-square test showed that there was a difference between the different age ranges of the patients with SSc in Western Paraná ($p < 0.0001$), showing a predominance in the 40-49 and 50-59 years age groups (61.4% of patients). The other characteristics analyzed (place of residence, time to diagnosis, and SSc form) showed a similarity between the patients, with no statistical difference.

Regarding the laboratory tests requested by the physicians, the ANA test was positive in 50 of the 57 patients (87.7%), and only one (1.75%) was negative, while six (10.5%) did not present this data in the medical record. Therefore, considering only the patients for whom this result was available, 98% had a reactive ANA. The data from this test are presented in Table 2. For the anti-ENA tests performed (Table 2), the anti-Scl 70 and ACA antibodies were reactive for 16 (28.1%) and 14 (25%) of the patients, respectively. These two autoantibodies showed more non-reactive (27 (47.4%) and 27 (47%), respectively) than reactive results.

When the ANA titers were analyzed, 38 patients (49.2%) were in the high titer range (1/640 and >1/640), with a significant difference from the other levels of titers observed in the patients ($p < 0.05$). Among the patterns reported for the ANA test, the most common was the centromeric pattern, which was seen in 26.3% of patients (15), followed by the nuclear fine speckled pattern (NFS) and the nuclear homogeneous pattern, representing 21.1% (12) and 17.5% (10) of the cases, respectively. Nuclear coarse speckled (NCS), nuclear dense fine speckled (NDFS), and nucleolar patterns were also observed (Table 3). The Chi-square test showed that the proportions of ANA patterns seen in patients diagnosed with SSc in Cascavel/PR differed significantly.

Table 1. Clinical and epidemiological characteristics of patients diagnosed with SSc in Cascavel/PR from March 2019 to September 2020.

Characteristics	n (57-100%)	p
Sex		<0.0001
Female	47 (82.5)	
Male	10 (17.5)	
Age		<0.0001
20-29	1 (1.8)	
30-39	8 (14.0)	
40-49	16 (28.1)	
50-59	19 (33.3)	
60-69	6 (10.5)	
70-79	2 (3.5)	
80-89	1 (1.8)	
Unreported	4 (7.0)	
Place of residence		0.7911
Cascavel	30 (52.6)	
Other cities	27 (47.4)	
Time to diagnosis		0.1676
Up to 6 months	16 (28.1)	
6 to 24 months	8 (14.0)	
More than 24 months	7 (12.3)	
Unreported	26 (45.6)	
Form		0.2673
Diffuse	30 (52.6)	
Limited	22 (38.6)	
Unreported	5 (8.8)	

SSc: Systemic sclerosis, p: p-value. The values highlighted in bold are statistically significant.

Table 2. Results of ANA and anti-ENA tests of patients diagnosed with SSc in Cascavel/PR from March 2019 to September 2020.

	Reactive	Non-reactive	UnR*	Total
	n (%)	n (%)	n (%)	n (%)
ANA	50 (87.7)	1 (1.75)	6 (10.5)	57 (100)
Anti-Scl 70	16 (28.1)	27 (47.4)	14 (24.6)	57 (100)
ACA	14 (25.0)	27 (47.0)	16 (28.0)	57 (100)
Anti-RNA polymerase III	3 (5.30)	15 (26.3)	39 (68.4)	57 (100)

*UnR: Unreported in the medical record, SSc – Systemic sclerosis, ANA – Antinuclear antibodies, ENA – Extractable nuclear antigens.

Table 3. Titers and patterns of positive ANA tests of patients diagnosed with SSc in Cascavel/PR from March 2019 to September 2020.

Characteristic	n (%)	p
ANA titer		0.0438
1/80	4 (7.0)	
1/160 to 1/320	17 (29.8)	
1/640	12 (21.1)	
>1/640	16 (28.1)	
Unreported	8 (14.0)	
ANA pattern		0.001
Nuclear homogeneous	10 (17.5)	
NFS	12 (21.1)	
NPG	3 (5.3)	
NDFS	4 (7.0)	
Centromeric	15 (26.3)	
Nucleolar	2 (3.5)	
Unreported	11 (19.3)	

SSc – Systemic sclerosis, ANA – Antinuclear antibodies, NFS – Nuclear fine speckled, NCS – Nuclear coarse speckled, NDFS – Nuclear dense fine speckled; p: p-value. The values highlighted in bold are statistically significant.

Discussion

The data found in patients with SSc in Cascavel and nearby regions are close to those reported in other countries. Comparison with other Brazilian data is challenging, as these are scarce. The predominance of female patients aged between 40 and 60 was also found by Lo Monaco, Bruschi, La Corte, Volpinari, & Trotta (2011) in Northern Italy, a predominantly Caucasian population comparable to the patients in this study (Lo Monaco et al., 2011). The female: male ratio in SSc is nearly 6, but it tends to decrease and approach 4 when there is a predominance of the diffuse form, which was found in Cascavel/PR (Claire & Cacoub, 2016). Patients diagnosed in western Paraná showed a predominance of the diffuse compared to the limited form (52.6 to 38.6%); this is generally not observed in SSc epidemiological studies, which usually report that most patients have the limited form (Barnes & Mayes, 2012).

The frequency of ANA positivity found in patients diagnosed with SSc in Cascavel/PR is in line with that found in the literature worldwide, with results above 90% (Stochmal et al., 2020). Tamaki, Mori, and Takehara (1991) detected the presence of ANA in 86% of those diagnosed with SSc in a study in Tokyo, Japan. In Brazil, in Campo Grande/MS, 94% positivity was found (Tamaki et al., 1991; Horimoto et al., 2016).

Although the ANA test is not a diagnostic criterion for SSc, the presence of specific autoantibodies is (Van Den Hoogen et al., 2013).Therefore, as practically all patients with SSc are positive for autoantibodies with Hep-2 as a substrate, the ANA test becomes an excellent screening method for the detection of these patients, given the ease of indirect immunofluorescence for the detection of these antibodies (Dahle, Skogh, Åberg, Jalal, & Olcén, 2004).

The presence of ACA antibodies (71.4%) in this study is similar to that seen in the literature. ACA antibodies occur in 55% to 80% of patients with limited SSc (Catoggio, Skinner, & Maddison, 1983; Mierau et al., 2011), with a higher prevalence in white patients and those over 50 years of age. Although highly associated with SSc, ACA antibodies are not entirely exclusive to this disease and can rarely be found in patients with primary biliary cirrhosis, Sjögren's syndrome, and systemic lupus erythematosus (SLE) (Chan, Lee, Hong, & Kuo, 1994; Pappas-Taffer, 2018). However, the presence of ACA in patients with isolated Raynaud's phenomenon may predict the future development of SSc (Ho & Reveille, 2003). Furthermore, the presence of these antibodies is strongly associated with pulmonary arterial hypertension (PAH) and severe digital ischemia (Herrick, 2018).

Andrade and Leser (2004) reported that the sensitivity of anti-Scl-70 antibodies in SSc is variable (26 to 76% of patients), depending on the population and methodology used (Andrade & Leser, 2004). In patients of the western region of Paraná, this percentage was 28.1%, slightly higher than another study carried out in southern Brazil, which found a prevalence of 17.8% (Skare, Fonseca, Luciano, & Azevedo, 2011). The diffuse form is linked to the presence of anti-Scl-70 antibodies. These antibodies are strongly associated with the onset of interstitial lung disease, renal crisis, and digital ulcers early in the condition (Stochmal et al., 2020).

The anti-RNA polymerase III antibodies in this study agree with other investigations that have detected them in 11% of patients with SSc, associated with diffuse skin involvement (Stochmal et al., 2020). In a retrospective study by Kuwana et al. (1999), anti-RNA polymerase III antibodies were associated with a worse prognosis. The associations described for these antibodies may be somewhat imprecise, as antibodies against more than one RNA polymerase often coincide in the same serum sample (Kuwana et al., 1999). The low availability of the test and the consequent small number of patients studied may also limit the definitive elucidation of the clinical significance of these autoantibodies.

In 2010, the EUSTAR group formulated preliminary criteria for the very early diagnosis of SSc, anticipating its diagnosis and treatment at an early stage of the disease. This consensus resulted in the definition of three-alarm signs for the very early diagnosis of SSc: the skin domain (e.g., swollen fingers), vascular domain (e.g., Raynaud's phenomenon), and laboratory domain (e.g., ANA reactivity) (Avouac et al., 2011). With these warning signs, the patient should be promptly referred for specialist evaluation. A later study corroborated the importance of this strategy: almost 90% of patients who presented the three warning signs presented autoantibodies specific to SSc or the pattern known as the 'scleroderma pattern' (SD) on nailfold capillaroscopy, fulfilling the criteria for the very early diagnosis of SSc proposed by EUSTAR (Minier et al., 2014). Thus, the need for the ANA and anti-ENA test is evident for SSc diagnosis to start treatment as soon as possible.

The patterns found in the results of the ANA test in this study, with a predominance of the centromeric pattern, are consistent with other studies, where this pattern shows specificity for SSc, especially in patients

with the limited form (Chan et al., 1994; Dellavance, Leser, & Andrade, 2007). The other patterns found, such as nuclear homogeneous and nuclear fine speckled, are also frequent in SSc (Mehra, Walker, Patterson, & Fritzler, 2013). In addition, a centromeric pattern in a positive ANA test is helpful in the diagnosis of SSc, as together with a nailfold capillaroscopy compatible with microangiopathy, particularly the SD pattern, it strongly predisposes to SSc diagnosis (Sampaio-Barros et al., 2013). These data confirm the ANA test results, predominant the centromeric pattern.

This study has some limitations, such as the incorrect filling out of medical records, leading to some cases of missing data, especially regarding specific antibodies associated with SSc. However, the main strength of this study is the addition of data to the scarce Brazilian literature about this disease.

Conclusion

The presence of autoantibodies in patients diagnosed with SSc in Cascavel/PR is within the worldwide range reported by the literature. This work is essential in adding data to the Brazilian literature, which is scarce in studies on SSc. These data should support the Brazilian guidelines for the diagnosis of SSc since, in the absence of national data, these guidelines are currently based on international information.

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