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Serological profile of patients with systemic sclerosis

Profil serologiczny chorych na twardzinę układową

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Summary

Introduction: The systemic sclerosis–associated autoantibodies include anti-centromere, anti-topoisomerase I (anti-topo I), anti-RNA polymerase III, anti-fibrillarin, anti-Th/To, and anti-PDGFR. A specific serological profile is connected with clinical manifestations and prognosis in systemic sclerosis (SSc).

Objectives: The aim of the study was to assess the serological profile in limited cutaneous and diffuse cutaneous SSc (lcSSc and dcSSc).

Patients and methods: 87 (68 female and 19 male) consecutive SSc patients treated between 2006 and 2011 were assessed. Patients fulfilled the American College of Rheumatology classification criteria of SSc: 35 – dcSSc and 52 – lcSSc. The following marker antibodies were determined: anti-topo I, anti-centromere A and B (CENP A, CENP B), anti-RNA polymerase III (RP11, RP 155), anti-fibrillarin (U3RNP), anti-NOR90, anti-Th/To, anti-PM-Scl-100, anti-PM-Scl-75, anti-Ku, anti-Ro-52, anti-PDGFR. The presence of antibodies was assessed using a test – EUROLINE Systemic Sclerosis Profile.

Results: 82 patients (94%) had positive antinuclear antibodies; anti-topo I – 29 patients; anti-CENP-A – 20 and anti-CENP-B – 20; anti-RP11 – 9 and anti-RP155 – 7; anti-U3RNP – 1; anti-NOR90 – 6; anti-Th/To – 3; anti-PM-Scl-100 – 7; anti-PM-Scl-75 – 11; anti-Ku – 5; anti-Ro-52 – 23 patients. We found significant differences in prevalence of anti-topo I: 25/35 vs. 4/52 p=0.0000; anti-CENP A: 0/35 vs. 20/52 p=0.0001; anti-CENP B: 0/35 vs. 20/52 p=0.0001 between dcSSc and lcSSc.

Conclusions: Some antibodies in SSc, e.g. anti-topo I and anti-centromere, are useful in defining the clinical subset of disease and provide prognostic information. There are no significant differences in the prevalence of other autoantibodies associated with SSc between dcSSc and lcSSc patients.

Key words: **systemic sclerosis • autoantibodies • serological profile**

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Abbreviations: **ANA** – antinuclear antibodies; **anti-CENP A, anti-CENP B** – anti-centromere A and B; **anti-RP11** and **anti-RP155** – anti-RNA polymerase III; **anti-topo I** – anti-topoisomerase I, anti-U3RNP, anti-fibrillarin; **dcSSc** – diffuse systemic sclerosis; **lcSSc** – limited systemic sclerosis; **SSc** – systemic sclerosis

Introduction

Autoimmunity is considered to be involved in the etiology and pathogenesis of systemic sclerosis (SSc). Antinuclear antibodies (ANAs) are present in 95-99% of patients with SSc [21, 20]. It is known that a specific serological profile is associated with clinical manifestations and prognosis in SSc. Anti-topoisomerase I (anti-topo I) and anti-centromere autoantibodies (ACAs) are the main antibodies connected with SSc. They are associated with a typical subset of disease and prognosis. ACAs are known to occur more often in limited cutaneous SSc (lcSSc), and are associated with better prognosis and late onset of visceral organ involvement. Anti-topo I antibodies are associated with diffuse cutaneous SSc (dcSSc), poorer prognosis and early onset of organ involvement [14, 6, 22]. The other SSc-associated autoantibodies include anti-RNA polymerase III, anti-PM-Scl, anti-Ku, anti-fibrillarin (anti-U3RNP), anti-Th/To, autoantibodies against nucleolus-organizing region-90 (anti-NOR90), anti-Ro52, and anti-PDGFR antibodies. These autoantibodies are less frequently described [6, 16]. The aim of the present study was to assess the entire serological profile in two groups of patients with SSc (lcSSc and dcSSc).

Patients and methods

The study included 87 SSc patients (68 female and 19 male) hospitalized consecutively in the Department of Rheumatology and Connective Tissue Diseases, Medical University of Lublin, Poland between 2006 and 2011. Patients fulfilled the American College of Rheumatology classification criteria for SSc. They were categorized according to the criteria of Le Roy et al. [10] as having lcSSc (n=52) or dcSSc (n=35). The mean age was 54.2 +/-12.7 years (range 21-80). The mean disease duration was 7.2+/-7.4 years (range 0.5-32) (Table 1). Serum samples were obtained from each patient. Subjects were examined for the presence of selected antinuclear antibodies (ANAs) such as anti-topo I, anti-centromere A and B (CENP A and CENP B), anti-RNA polymerase III (RP 11 and RP 155), anti-fibrillarin (U3RNP), anti-NOR90, anti-Th/To, anti-PM-Scl-100, anti-PM-Scl-75, anti-Ku, anti-Ro-52, and anti-PDGFR. The presence of antibodies was assessed using a commercial test, EUROLINE Systemic Sclerosis Profile, which is used to determine antibodies against SSc-specific antigens. Detection and interpretation of results was carried out electronically using the specific program EUROLINEScan (Euroimmun). All calculations were performed with Statistica 6.0. Data were analyzed using the chi-squared test (variation test) for comparison between groups. P values < 0.05 were considered statistically significant.

Results

According to our observations, 82 patients (94.25%) had positive ANAs; anti-topo I was detected in 29 (33.33%) patients, anti-CENP A in 20 (23.0%), anti-CENP B in 20 (23.0%), anti-RP11 in 9 (10.34%), anti-RP155 in 7 (8.04%), anti-U3RNP in 1 (1.14%), anti-NOR90 in 6 (6.9%), anti-Th/To in 3 (3.44%), anti-PM-Scl-100 in 7 (8.04%) and anti-PM-Scl-75 in 11 (12.64%) patients; anti-Ku was detected in 5 (5.74%) and anti-Ro-52 in 23 (26.43%) subjects. There were no anti-PDGFR antibodies observed (Table 2). The differences in prevalence of ANAs between dcSSc and lcSSc groups were 34/35 vs. 48/52 and were not statistically significant. The prevalence of anti-topo I antibodies was statistically significantly higher in the dcSSc compared to the lcSSc group: 25/35 vs. 4/52 p=0.0000. Moreover, the prevalence of anti-CENP A and anti-CENP B antibodies was statistically significantly higher in the lcSSc group compared to the dcSSc group: 20/52 vs. 0/35 p = 0.0001 and 20/52 vs. 0/35 p = 0.0001. There were no statistically significant differences observed in the prevalence of anti-RP11, anti-RP155, anti-U3RNP, anti-NOR90, anti-Th/To, anti-PM-Scl-100, anti-

Table 1. Characteristics of the study group

	Clinical data
Number of patients Women Men	87 68 19
Type of SSc (number of patients)	dcSSc – 35 lcSSc – 52
Mean age (years)	53.58+/-13.52
Mean disease duration (years)	7.2+/-7.4

Table 2. The prevalence of antibodies associated with SSc in SSc group

AUTOANTIBODIES	SSc GROUP (n=87)
ANA	82 (94.25 %)
anti- topo I	29 (33.33%)
anti-CENP A	20 (23.0%)
anti-CENP B	20 (23.0%)
anti-RP11	9 (10.34%)
anti-RP 155	7 (8.04%)
anti-U3RNP	1 (1.14%)
anti-NOR90	6 (6.9%)
anti-Th/To	3 (3.44%)
anti-PM-Scl-100	7 (8.04%)
anti-PM-Scl-75	11 (12.64%)
anti-Ku	5 (5.74%)
anti-Ro-52	23 (26.43%)
anti-PDGFR	0

Table 3. The differences in prevalence of SSc-associated antibodies between dcSSc vs lcSSc

	dcSSc (pts/35)	lcSSc (pts/52)	P<0.05
ANA	34/35	48/52	NS
anti-topo I	25/35	4/52	p=0.0000
anti-CENP A	0/35	20/52	p=0.0001
anti-CENP B	0/35	20/52	p=0.0001
anti-RP 11	5/35	4/52	NS
anti-RP155	5/35	5/52	NS
anti-U3RNP	1/35	0/52	NS
anti-NOR90	1/35	5/52	NS
anti-Th/To	1/35	2/52	NS
anti-PM-Scl-100	3/35	4/52	NS
anti-PM-Scl-75	2/35	9/52	NS
anti-Ku	2/35	3/52	NS
anti-PDGFR	0/35	0/52	NS
anti-Ro-52	8/35	15/52	NS

-PM-Scl-75, anti-Ku, anti-PDGFR and anti-Ro-52 antibodies between dcSSc and lcSSc patients (Table 3).

Discussion

The use of antibodies to assist in the diagnosis, classification and prognostication of SSc has been reviewed elsewhere [17]. It is known that ANAs are present in the sera of lcSSc and dcSSc patients. The diagnostic sensitivity of various assays for ANAs in SSc varies from 75% to 95% [6, 19]. According to our results, ANAs were detected in 94% of SSc patients. There were no significant differences in the prevalence of ANAs between dcSSc and lcSSc groups. Anti-topo I and ACAs are classically associated with SSc, and respectively demonstrate nucleolar/homogeneous and centromeric staining patterns on ANA screening by immunofluorescence [13]. Anti-topo I antibodies are 100% specific for SSc compared with normal control groups, and 99.5% specific compared with the group of patients with other connective tissue diseases [17]. According to different assays, including immunodiffusion, immunoblotting, and enzyme-linked immunosorbent assay, the prevalence

of anti-topo I antibodies varies from 15% to 40% or even to 65% [6, 17, 3]. Our findings show the presence of anti-topo I in 33% of SSc patients according to the immunoblotting method. Patients with anti-topo I antibodies frequently develop pulmonary fibrosis, peripheral vascular disease, cardiac involvement, scleroderma renal crises and malignancies. The literature data demonstrate that anti-topo I antibodies are associated with high mortality rates [6, 3]. The recent data show that these antibodies may be present in sera of patients with systemic lupus erythematosus (SLE) [12]. Moreover, anti-topo I antibodies are more frequent in dcSSc and their prevalence varies from 30% to 40%, compared to less than 10% in lcSSc [6, 17, 3]. In our study, anti--topo I antibodies were found in 71% of dcSSc patients and in 8% of lcSSc patients. Such a high anti-topo I prevalence in dcSSc might have resulted from high frequency of severe cases with more advanced diseases, who were hospitalized in our department. According to the literature, anti-CENP--A and anti-CENP-B are found in 20-40% of patients with SSc but the prevalence in lcSSc ranges from 40 to 90% and depends on the method of assessment used [3]. Higher risk for digital gangrene and amputation is noted in the anticentromere-positive group. Pulmonary vascular disease with pulmonary arterial hypertension (PAH) and right heart failure occur in a significant proportion of patients. A factor particularly associated with poor prognosis in lcSSc is PAH. Anti-centromere antibodies are associated with limited skin involvement, peripheral vascular damage and calcinosis. They have also been reported in patients with SLE, primary biliary cirrhosis, rheumatoid arthritis and Sjögren's syndrome. [6, 17, 13, 7]. According to our observations, the prevalence of ACAs was comparable to the reported literature data and was significantly higher in lcSSc patients. RNA polymerase III antibodies occur in 5-20% of SSc patients [18, 11] and are associated with diffuse cutaneous involvement, renal crisis and a more progressive course of the disorder [9, 4, 15, 8]. According to our observations, their prevalence was about 10% in SSc patients. Anti-fibrillarin antibodies are strongly associated with dcSSc, but they are seen in less than 10% of SSc patients [6, 5]. In our study, these antibodies were present in about 1% of SSc and no significant differences were noted between the subgroups; however, the study group was small. Anti-Th/To and anti-PM/Scl antibodies are associated with lcSSc [6, 2]. The prevalence of anti-PM/Scl antibodies in SSc ranges from 4 to 11%. They are found in approximately 25% of SSc patients with myositis overlap and in 2% of patients with SSc alone [6]. In our study, anti-PM-Scl-100 antibodies were detected in 8% and anti-PM-Scl-75 in 13% of SSc cases. We excluded the overlap syndrome but 5 patients in this group had muscle pain and 3 had elevated levels of CK. These patients did not fulfill the criteria of polymyositis. The other antibodies connected with PM/SSc overlap syndrome are anti-Ku. They have been reported in sera from patients with other connective tissue diseases such as SLE and other overlap syndromes [6]. Their prevalence in SSc is rather low [1]. In our study group, overlap was excluded. Anti-Th/To antibodies are present in a small subset of patients with SSc (2-5%) and are associated with limited skin involvement [6]. According to our data, anti-Th/To antibodies were present in 3.5% of SSc patients. Moreover, no significant differences in their prevalence were found between the types of disease; however, the group studied was small. Anti-NOR-90 antibodies were initially described in 1987. They are not SSc-specific and are also found in other connective tissue diseases such as Raynaud's disease, rheumatoid arthritis, SLE and in malignancies [6]. The prevalence of anti-PDGFR and anti-Ro52 antibodies in SSc is rather low and their role requires further investigations. In our study, the prevalence of the latter was high. Both groups of antibodies are not specific for SSc and they are found in SLE, Sjögren's syndrome, rheumatoid arthritis and idiopathic pulmonary fibrosis. Furthermore, anti-PDGFR antibodies may have a pathogenic role in SSc, as PDGFR expression is increased by pathological transforming growth factor-beta signaling and binding of PDGFR to anti-PDGFR, leading to overproduction of collagen [6, 16].

In conclusion, both literature data and our observations demonstrate that some antibodies in SSc, such as anti-topo I and anti-centromere antibodies, are useful in defining the clinical subset of disease and provide prognostic information. There are no significant differences in the prevalence of other autoantibodies associated with SSc between dcSSc and lcSSc patients. According to the literature, anti-PM/Scl antibodies are associated with myositis in the course of SSc. Our observations confirmed these data. Many autoantibodies associated with SSc can be found in SSc patients but their role is unknown. The literature data concerning the role of these rare antibodies are not numerous, and most of them are case reports. In our study, the high incidence of anti-Ro antibodies in SSc patients is of interest. Their influence on the clinical prognosis in SSc requires further research.

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The authors have no potential conflicts of interest to declare.