Clinical and Autoantibody Associations in Antinuclear Antibody–Positive Systemic Sclerosis Lacking Prototypic Autoantibodies

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Systemic sclerosis (SSc) is a fibrotic disease that is clinically, immunologically, and molecularly heterogeneous.[1] Ninety-five percent of patients have antinuclear antibodies (ANA), and most have prototypic SSc-associated antibodies including anticentromere (ACA), anti–Scl-70 (ATA), or anti-RNA polymerase-III (RNAP3), each which has strong clinical associations and is predictive of outcomes.[2] Additional SSc-related antibodies including fibrillarin and Th/To have been identified but are not routinely tested. Because SSc diagnostic criteria do not require specific autoantibodies, clinicians may frequently encounter patients who meet the diagnostic criteria clinically but who are negative for all 3 prototypical SSc autoantibodies ("triple-negative"). Unlike each of the well-characterized antibody subsets, clinical associations and outcomes for these triple-negative patients are not well characterized.

The purposes of this study were to identify ANA-positive and triple-negative SSc patients and assess their demographic and clinical characteristics. In addition, we sought to investigate the presence of other autoantibodies in this subgroup and determine clinical associations.

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MATERIALS AND METHODS

Study Population

Patients from University of Rochester Medical Center (URMC) and Northwestern University (NU) scleroderma repositories were evaluated. The institutional review board of the URMC approved this case series (RSRB #71768). This research was in compliance with the Helsinki Declaration. All participants gave written informed consent to participate. Inclusion criteria included age older than or equal to 18 years, fulfillment of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) SSc diagnostic criteria, and ability to provide informed consent.[3] Exclusion criteria included patients who were diagnosed with another rheumatologic disease including mixed connective tissue disease. Patients with an overlap connective tissue disease were not excluded if they met the ACR/EULAR SSc criteria. Northwestern University patients fulfilled the same criteria and were drawn from a prior study.[4]

Clinical Characteristics

Demographic information and clinical data were obtained from chart review and recorded from the time of first SSc clinic appointment at which point blood was drawn for autoantibody testing. Patients were characterized by disease subset and modified Rodnan skin score (MRSS) at the initial SSc visit. Presence of digital ulcers, telangiectasias, and interstitial lung disease (ILD) on chest computed tomography (honeycombing, ground-glass opacities) was evaluated, with positivity documented at any point in time since the initial visit. Pulmonary arterial hypertension (PAH) was assessed by right-sided heart catheterization, and maximum pressures were recorded. Maximum creatine kinase (CK) scores were documented.

Immunofluorescence and Immunoblot

Sera were screened for ANA by indirect immunofluorescence (IIF) on HEp-20-10 slides, and fluorescence intensity, pattern, and titer were evaluated by the EUROPattern microscope and software (EUROLabPicture; EUROIMMUN, Lübeck, Germany).[5] Autoantibody confirmation was performed using immunoblots (EUROLINE SSc Profile 12 Ag [immunoglobulin G]; autoimmune inflammatory myopathies 16 Ag et cN-1A; SSc Profile [Nucleoli]; EUROIMMUN US, Mountain Lakes, NJ).[5]

Positive and negative controls were used to identify the intensity of each reactivity with antibody results reported as follows: 0 (negative), + (borderline positivity), ++ (positive), +++ (strongly

positive). No differences were noted between borderline and positive results on data stratification; thus, both were included.

Statistical Analysis

Demographic and clinical parameters were expressed as mean ± SD, whereas categorical results were expressed as frequencies. Clinical associations between antibodies and phenotype were assessed using Fisher's exact test. Clinical associations between the number of positive antibodies and phenotype were assessed using Student's *t* test. For each test, $p < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Using standard clinical laboratory testing, 57 patients (20.4%) were identified as ANA-positive, triple-negative SSc, including 45 of 200 patients (22.5%) from the NU cohort and 12 of 80 patients (15%) from the URMC cohort. Study population characteristics are summarized in Table 1.

Of the 57 triple-negative SSc patients initially identified, 40 were confirmed by immunoblot. Of these, 33 were women (82.5%), and patients were primarily White (75.0%), with a mean age of 53.0 ± 14.5 years. Patients had a similar distribution of limited and diffuse cutaneous disease (52.5% vs 37.5%) with an average MRSS of 7.6 ± 6.8. Telangiectasia (72.5%) and digital ulcers (47.5%) were highly prevalent. The majority of patients (60.0%) had ILD, and 15% had PAH. Average forced vital capacity (FVC) was 79.0% ± 20.6% predicted, and diffusing capacity of lungs for carbon monoxide (DLCO) was 62.0% ± 19.5% predicted. Fourteen patients (35%) had elevated CK with an average CK level of 152.5 ± 162.1 U/L. No patients developed renal crisis. One patient had overlap with polymyositis and Sjögren, and 1 patient had rheumatoid arthritis overlap.

Antibody Prevalence

Antinuclear antibody was confirmed by IIF in all patients, with a mixed speckled/nucleolar (42.5%) and speckled (30.0%) patterns being most prevalent. Table 2A depicts the 29 antibodies assessed by immunoblot and categorization of prototypic scleroderma antibodies (ACA, ATA, RNAP3), scleroderma-associated antibodies, and myositis antibodies (MAA) defined by the EUROIMMUN.[5] Supplemental Diagram 1 (http://links.lww.com/RHU/A471) depicts the study population flow diagram and exclusion of the 17 prototypic autoantibodies. Antibody prevalence as measured by immunoblot is described in Table 2B and Supplemental Table 2 (http://links.lww.com/RHU/A472). The most prevalent antibody detected was Ro-52 (50%). Ro-52 positivity was

TABLE 1. Clinical Characteristics of Cohort

Variables	NU (n = 30)	URMC (n = 10)	Combined (n = 40)
Demographics			
Female	25 (83.33%)	8 (80)	33 (82.5)
Age, y	47 ± 10.81	69 ± 11.39	53 ± 14.49
White	20 (66.67)	10 (100)	30 (75)
Hispanic	5 (16.67)	0 (0)	5 (12.5)
African American	4 (13.33)	0 (0)	4 (10)
Asian/Pacific Islander	1 (3.33)	0 (0)	1 (2.5)
ANA pattern			
Centromere	0 (0)	0 (0)	0 (0)
Cytoplasmic	6 (20)	2 (20)	8 (20)
Homogenous	3 (10)	0 (0)	3 (7.5)
Nucleolar	12 (40)	7 (70)	19 (47.5)
Partly nucleolar	1 (3.33)	0 (0)	1 (2.5)
Speckled	26 (86.67)	9 (90)	35 (87.5)
Subtypes			
Limited cutaneous SSc	14 (46.67)	7 (70)	21 (52.5)
Diffuse cutaneous SSc	14 (46.67)	1 (10)	15 (37.5)
Overlap	1 (3.33)	1 (10)	2 (5)
SSc sine scleroderma	2 (6.67)	1 (10)	3 (7.5)
Disease characteristics			
Average disease duration	6 ± 5.67	18.8 ± 13.14	9 ± 9.73
Telangiectasias	22 (73.33)	7 (70)	29 (72.5)
Digital ulcers	16 (53.33)	3 (30)	19 (47.5)
Average MRSS	8.13 ± 7.35	6 ± 4.85	7.55 ± 6.83
ILD	20 (66.67)	4 (40)	24 (60)
PAH	5 (16.67)	1 (10)	6 (15)
Average FVC (range)	74 ± 16.92 (21–102)	97 ± 24.44 (50–129)	79 ± 20.55 (21–129)
Average DLCO (range)	61 ± 18.37 (19–89)	63 ± 24.67 (21–102)	62 ± 19.53 (19–102)
Average CK (range)	159.44 ± 164.49 (31–871)	129 ± 162.17 (31–524)	152.54 ± 162.08 (31–871)

Values are presented as frequencies (percentages) or mean ± SD.

TABLE 2. Description of EUROIMMUN Immunoblot Antibody Panels Assessing 29 Autoantibodies Assessed in the Triple-Negative Cohort (n = 40) (A) and Antibody Prevalence by Immunoblot (B)

A.

Scleroderma panel	Scl-70, CEN-A, CEN-B, RP11, RP155, Th/To, fibrillarin, NOR-90, RNP-A, RNP-C, RNP-70, PDGFR, Ro-52
Myositis panel	MDA5, SAE1, Mi-2b, PM75, PM100, Ku, SRP, CN-1a, NXP2, PL-7, Jo1, Mi-2a, PL-12, T1F1g, OJ, EJ

B.

Antibody	Prevalence n (%)
Ro-52	20 (50)
Th/To	16 (40)
MDA5	14 (35)
SAE1	11 (27.5)
Fibrillarin	10 (25)
Ku	9 (22.5)
Mi-2b	8 (20)
PM75/PM100	6 (15)

A: Prototypic scleroderma antibodies: Scl-70, CEN-A, CEN-B, RP11, RP155. Scleroderma-associated antibodies: Th/To, Fibrillarin, NOR-90, RNP-A, RNP-C, RNP-70. Other antibodies included PDGFR, Ro-52. Myositis antibodies: MDA5, SAE1, Mi-2b, PM75, PM100, Ku, SRP, CN-1a, NXP2, PL-7, Jo1, Mi-2a, PL-12, T1F1g, OJ, EJ. B: Antibodies with a prevalence of less than 20% are not shown (n = 20), with exception of PM75/PM100, which individually had a prevalence of greater than 20% but are listed together as the antibodies are clinically meaningful when double-positive.

significantly associated with prevalence of ILD (relative risk [RR], 2.67; $p = 0.0007$) and elevated CK (RR, 2.64; $p = 0.04$). Among the scleroderma-associated antibody group, Th/To (40%) and fibrillarin (25%) were the most prevalent, followed by NOR-90, RNP-A, and RNP-C (7.5% each), but none were associated with specific clinical manifestations. The most common MAA were MDA5 (35%) and SAE1 (27.5%). Mi-2b (20%) and PM-75 (25%) were less common, but were significantly associated with MRSS >12 (Mi-2b RR, 4.00; $p = 0.04$) and digital ulcers (PM-75 RR, 2.18; $p = 0.03$). Antibody associations with clinical outcomes are detailed in Table 3 and Supplemental Table 3 (http://links.lww.com/RHU/A473).

Many patients were positive for multiple autoantibodies (mean, 3.68 ± 2.3), and this was associated with increased severity of pulmonary function test parameters. A Student's *t* test was used to assess clinical significance. Patients with 3 or more antibodies (n = 30) had lower FVC scores compared with patients with 0 to 2 antibodies (n = 10, $p = 0.005$) and lower DLCO scores ($p = 0.02$) (Supplemental Table 4, http://links.lww.com/RHU/A474).

DISCUSSION

The vast majority of research in SSc characterizes clinical features of patients on the basis of antibody subsets (Scl-70, centromere, and RNA polymerase-III) without specifically characterizing patients who are ANA-positive but triple-negative. Whereas some studies have looked at patients who were ANA-negative triple-negative, this study represents the first multicenter characterization of this relatively prevalent ANA-positive, triple-negative subgroup (14% of SSc patients). We identified 40 patients who met these parameters and were able to detect autoantibodies in the majority of these patients by immunoblot. Patients had a high prevalence of ILD and myositis-specific antibodies (MSA)/MAA,[6] and importantly, some antibodies were associated with specific clinical manifestations. In addition, patients with increased numbers of autoantibodies (≥3) were associated with more severe ILD.

Most SSc case series extensively characterize patients with Scl-70, centromere, and RNA polymerase III antibodies, but lack description of patients who are negative for these. Patients in this study were included based on the ACR/EULAR criteria, which accounts only for the prototypic SSc antibodies. For future diagnostic purposes, inclusion of any SSc-specific antibody (not limited to the 3 prototypic antibodies) may allow for a more inclusive SSc definition and should be considered.

Only 3 studies primarily focused on ANA-negative patients reported on the ANA-positive triple-negative SSc group.[7–9] Miyake et al.[7] identified 5.3% of patients were ANA-positive triple-negative SSc with median MRSS of 13, ILD in 62%, and pulmonary hypertension in 3%. Hudson et al.[8] found only 1.8% of 874 patients to be ANA-positive but extractable nuclear antigen antibodies (ENA)-negative and reported a lower prevalence of ILD (12.5%). Liu et al.[9] identified that telomere length was shorter in triple-negative SSc patients in comparison to patients with prototypic antibodies, and this was associated with ILD and increased risk for deterioration in lung function. Compared with these studies from Japan and Canada, the prevalence of SSc patients who were ANA-positive triple-negative was significantly higher in our Fisher'scombined US cohort at 14%. Although we observed a higher prevalence of these patients, we found similar clinical features, with 60% of patients with ILD and mild skin involvement (MRSS, 7.55).

Of the 29 antibodies tested by immunoblot, patients in our 2 cohorts showed positivity for 26, with the highest prevalence seen for Ro-52, Th/To, and MDA5. Routine clinical autoantibody testing failed to detect patients with weakly positive SSc-associated antibodies, and 17 patients (6%) were initially classified as triple-negative SSc based on routine clinical testing. These patients were reclassified as positive for one of the prototypic antibodies after immunoblot, which

TABLE 3. Assessment of Autoantibodies and Associated Significant Clinical Outcomes

Antibody	ILD			MRSS >12			CK (>145)			Digital Ulcers		
	n (%)	RR (95% CI)	*p* value	n (%)	RR (95% CI)	*p* value	n (%)	RR (95% CI)	*p* value	n (%)	RR (95% CI)	*p* value
Ro-52	**17 (85)**	**2.67 (1.51–5.29)**	**0.0007**	4 (20)	1 (0.30–3.25)	>0.99	**10 (50)**	**2.64 (1.11–6.96)**	**0.04**	11 (55)	1.37 (0.71–2.74)	0.53
PM75	6 (60)	1.10 (0.54–1.84)	>0.99	2 (20)	1.00 (0.25–3.50)	>0.99	4 (40)	1.00 (0.38–2.22)	>0.99	**8 (80)**	**2.18 (1.17–3.85)**	**0.03**
Mi-2b	5 (63)	1.12 (0.52–1.85)	>0.99	**4 (50)**	**4.00 (1.25–11.75)**	**0.04**	2 (25)	0.67 (0.18–1.78)	0.68	5 (63)	1.43 (0.65–2.56)	0.44

For each clinical outcome (ILD, MRSS, CK, and digital ulcers), patients were stratified by autoantibody, and associations were determined using a Fisher's exact test. Prevalence of each clinical feature was calculated based on antibody prevalence. Statistically significant results ($p < 0.05$) are in bold font.

CI, confidence interval.

has improved sensitivity. The distinction between clinical and immunoblot testing shows that the definition of "triple-negative SSc" patients depends on the testing used.

Anti–Ro-52 (TRIM21) was the most prevalent antibody in our triple-negative SSc patients (50%). Hudson et al.[10] previously reported Ro-52 antibodies in 20% of a large cohort of SSc patients, which were associated with ILD (odds ratio, 1.53) and overlap syndrome including 11.5% of patients with Ro-52, demonstrating inflammatory myositis. In comparison, we found Ro-52 in 50% of our ANA-positive triple-negative cohort with an increased ILD association (RR, 2.67). Although many patients had ENA antibody testing clinically, we did not specifically assess anti-Ro/SSA or anti-La/SSB as part of the immunoblot panel, and this full characterization may be useful in future studies.

Although not an MSA, Ro-52 is highly prevalent in myositis patients and frequently co-occurs with antisynthetase antibodies.[11] Moreover, the coexistence of SSc and myositis/myopathy identifies patients with poor prognosis[12]; thus, we investigated patient sera for a variety of MSA/MAA. Multiple MSA/MAA (n = 15) were observed in the triple-negative SSc cohort and classified as described by Leurs et al.[6] We found the prevalence of MSA at 32.5% and MAA at 30%. These proportions are elevated in the triple-negative SSc patients compared with the literature, where 8% had MSA and 9.7% had MAA in a European SSc cohort. No patients with myositis antibodies also fulfilled the EULAR/ACR inflammatory myopathy classification criteria, suggesting that myositis patients were not misclassified as having SSc. We also found a high proportion of patients positive for anti-MDA5 antibodies compared with previous reports.[6] Because the majority of the MDA5 titers were low, and these patients did not demonstrate classic MDA5 clinical presentations such as rapidly progressive ILD or digital ulcerations,[13] further testing should be performed to confirm these positive results. Among the less prevalent MSA/MAA, we also found an association between Mi-2b and elevated MRSS and an association between PM-75 antibodies and digital ulcers. Mi-2b is frequently identified in dermatomyositis, but there is a paucity of data on clinical correlates of this autoantibody in SSc. Wodkowski et al.[14] described associations between PM-75 and calcinosis but not ulceration.

This study was limited in its power to detect associations between individual autoantibodies and clinical manifestations because of the relatively low prevalence of triple-negative SSc patients and each of the specific antibodies. Further studies are needed to confirm our results in larger populations and to assess additional clinical parameters such as nailfold capillaroscopy. The autoantibodies assessed are not exhaustive, and additional valuable information could be assessed through testing for more recently described novel antibodies.[15] The sensitivity and specificity of individual assays should also be compared with criterion-standard assays, such as immunoprecipitation assays, as commercial autoantibody assays are not always accurate. This validation is essential when IIF shows patterns inconsistent with results obtained by commercial immunoassay and can further characterize potential nonspecific positive results. The sensitivity and specificity of the immunoblot have not been compared with other commercially available antibody tests in this population. Another limitation is that although the high prevalence of myositis antibodies is important, we only characterized CK levels as the study did not have standardized clinical data to indicate weakness or other objective findings of myositis. Future studies should assess Ro-52 and myositis antibodies in triple-negative versus other SSc populations.

Performing extended antibody panel testing such as the immunoblot panel used in this study is rational in patients with clinical triple-negative SSc. Knowledge of specific autoantibodies (Ro-52, PM-75, Mi-2b) should make the clinician consider more diligent screening. Ro-52 (SS-A) is part of the standard ENA panel and certainly should be assessed in all SSc patients given its association with ILD. These patients should be more closely monitored with baseline chest computed tomography and frequent pulmonary function tests. In patients with MSA/MAA, additional focus should be given to assessing potential muscle disease including a complete neurologic examination with manual muscle testing, assessment of muscle enzymes, and consideration of an electromyography or magnetic resonance imaging. Because the extended immunoblot antibody profile is not routinely available, clinicians may consider ordering a myositis antibody panel and additional SSc serologies in triple-negative patients.

In conclusion, ANA-positive patients who are negative for prototypic SSc antibodies have a high prevalence of Ro-52 antibodies, an enrichment for MSA, and increased risk of ILD. These patients are seen relatively frequently (14% of SSc patients in this cohort) and should be regularly assessed for evidence of myopathy and lung involvement.

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REFERENCES

1. Mehra S, Walker J, Patterson K, et al. Autoantibodies in systemic sclerosis. *Autoimmun Rev*. 2013;12:340–354. doi:10.1016/j.autrev.2012.05.011.
2. Hamaguchi Y. Autoantibody profiles in systemic sclerosis: predictive value for clinical evaluation and prognosis. *J Dermatol*. 2010;37:42–53. doi:10.1111/j.1346-8138.2009.00762.x.
3. van den Hoogen F, Khanna D, Fransen J, et al. 2013 Classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis*. 2013;72:1747–1755. doi:10.1136/annrheumdis-2013-204424.
4. Korman BD, Marangoni RG, Hinchcliff M, et al. Brief report: association of elevated adipsin levels with pulmonary arterial hypertension in systemic sclerosis. *Arthritis Rheumatol*. 2017;69:2062–2068. doi:10.1002/art.40193.
5. Liaskos C, Marou E, Simopoulou T, et al. Disease-related autoantibody profile in patients with systemic sclerosis. *Autoimmunity*. 2017;50:414–421. doi:10.1080/08916934.2017.1357699.
6. Leurs A, Dubucquoi S, Machuron F, et al. Extended myositis-specific and -associated antibodies profile in systemic sclerosis: a cross-sectional study. *Joint Bone Spine*. 2021;88:105048. doi:10.1016/j.jbspin.2020.06.021.
7. Miyake M, Matsushita T, Takehara K, et al. Clinical features of Japanese systemic sclerosis (SSc) patients negative for SSc-related autoantibodies: a single-center retrospective study. *Int J Rheum Dis*. 2020;23:1219–1225. doi:10.1111/1756-185X.13908.
8. Hudson M, Satoh M, Chan JY, et al. Prevalence and clinical profiles of 'autoantibody-negative' systemic sclerosis subjects. *Clin Exp Rheumatol*. 2014;32(6 Suppl 86):S-127-32.
9. Liu S, Chung MP, Ley B, et al. Peripheral blood leucocyte telomere length is associated with progression of interstitial lung disease in systemic sclerosis. *Thorax*. 2021;76:1186–1192. doi:10.1136/thoraxjnl-2020-215918.
10. Hudson M, Pope J, Mahler M, et al. Clinical significance of antibodies to Ro52/TRIM21 in systemic sclerosis. *Arthritis Res Ther*. 2012;14:R50. doi:10.1186/ar3763.
11. Rutjes SA, Vree Egberts WT, Jongen P, et al. Anti-Ro52 antibodies frequently co-occur with anti–Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy. *Clin Exp Immunol*. 1997;109:32–40. doi:10.1046/j.1365-2249.1997.4081308.x.
12. Paik JJ, Wigley FM, Shah AA, et al. Association of fibrosing myopathy in systemic sclerosis and higher mortality. *Arthritis Care Res (Hoboken)*. 2017;69:1764–1770. doi:10.1002/acr.23291.

13. Moghadam-Kia S, Oddis CV, Aggarwal R. Anti-MDA5 antibody spectrum in Western world. *Curr Rheumatol Rep*. 2018;20:78. doi:10.1007/s11926-018-0798-1.

14. Wodkowski M, Hudson M, Proudman S, et al. Clinical correlates of monospecific anti-PM75 and anti-PM100 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects. *Autoimmunity*. 2015;48:542–551. doi:10.3109/08916934.2015.1077231.

15. Mecoli CA, Casciola-Rosen L. An update on autoantibodies in scleroderma. *Curr Opin Rheumatol*. 2018;30:548–553. doi:10.1097/BOR.0000000000000550.