Clinical and Autoantibody Associations in ANA-Positive Systemic Sclerosis Lacking Prototypic Autoantibodies

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Introduction

- Systemic sclerosis (SSc) is a fibrotic disease which is clinically, immunologically, and molecularly heterogeneous.

- The majority of patients have positive anti-nuclear antibodies (ANA) and most have prototypic SSc-associated antibodies including anti-centromere (ACA), anti-Scl-70 (ATA), or anti-RNA polymerase-III (RNAP3), each of which has strong clinical associations and are predictive of outcomes.

- The subset of ANA-positive patients with SSc who lack prototypic autoantibodies has been poorly characterized.

- The purpose of this study was to:

  - Identify ANA positive and triple negative SSc patients and assess their demographic and clinical characteristics.

  - Investigate the presence of specific autoantibodies not routinely clinically assayed and determine their clinical associations.

Materials & Methods

Study Population & Clinical Characteristics

- 280 patients from the URMC and Northwestern scleroderma registries with available serum were evaluated for clinical autoantibody status.

- Patients were assessed for disease subset, skin involvement (modified Rodnan skin score), digital ulcers, telangiectasias, CK levels, interstitial lung disease (ILD) on chest CT (as defined by honeycombing, ground glass opacities, and reticulation), and pulmonary arterial hypertension (PAH) as assessed by right heart catheterization.

Immunofluorescence and Immunoblot

- Sera from 57 clinically triple negative patients were screened for ANA by indirect immunofluorescence (IIF) on HEp-20-10 slides (Figure 1).

- 29 additional autoantibodies were assessed using EUROLINE SSc and inflammatory myopathy immunoblots.

- Positive and negative controls were used to identify the intensity of each reactivity with antibody results reported on a scale as: 0 (negative), + (borderline positivity), ++ (positive), +++ (strongly positive). Borderline positivity was considered positive.

Results

Patient Characteristics (Table 1)

- Forty (14%) ANA+ triple negative SSc patients were confirmed by immunoblot.

- 17 patients who were initially thought to be triple negative were identified as having anti-centromere, anti-topoisomerase, or anti-RNA polymerase III.

- Speckled and mixed speckled/nucleolar were the most common ANA patterns.

- Triple negative patients had similar prevalence of limited and diffuse SSc.

- There was high prevalence of digital ulcers (48%), elevated CK (35%), ILD (60%), and a low burden of skin disease (MRSS was 7.6 ± 6.8).

Results

Variables	NU (n=30) Frequency (%)	URMC (n=10) Frequency (%)	Combined (n=40) Frequency (%)
Demographics			
Female	25 (83.33%)	8 (80)	33 (82.5)
Age, ± mean SD years	47 ± 10.81	69 ± 11.39	53 ± 14.49
Caucasian	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	14 (46.67)	7 (70)	21 (52.5)
Diffuse	14 (46.67)	1 (10)	15 (37.5)
Overlap	1 (3.33)	1 (10)	2 (5)
Sine	2 (6.67)	1 (10)	3 (7.5)
Disease Characteristics			
Avg disease duration ± SD	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)
Avg MRSS ± SD (Range)	8.13 ± 7.35 (1-25)	6 ± 4.85 (0-18)	7.55 ± 6.83 (0-25)
ILD	20 (66.67)	4 (40)	24 (60)
PAH	5 (16.67)	1 (10)	6 (15)
Avg FVC ± SD (Range)	74 ± 16.92 (21-102)	97 ± 24.44 (50-129)	79 ± 20.55 (21-129)
Avg DLCO ± SD (Range)	61 ± 18.37 (19-89)	63 ± 24.67 (21-102)	62 ± 19.53 (19-102)
Avg CK ± SD (Range)	159.44 ± 164.49 (31-871)	129 ± 162.17 (31-524)	152.54 ± 162.08 (31-871)

Table 1. Clinical characteristics of cohort. Table depicts frequencies (percentages), or mean ± SD and range. NU: Northwestern University; URMC: University of Rochester Medical Center; ANA: anti-nuclear antibodies; MRSS: modified Rodnan skin score; CK: creatine kinase; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; FVC: forced vital capacity; DLCO: diffusing capacity for carbon monoxide.
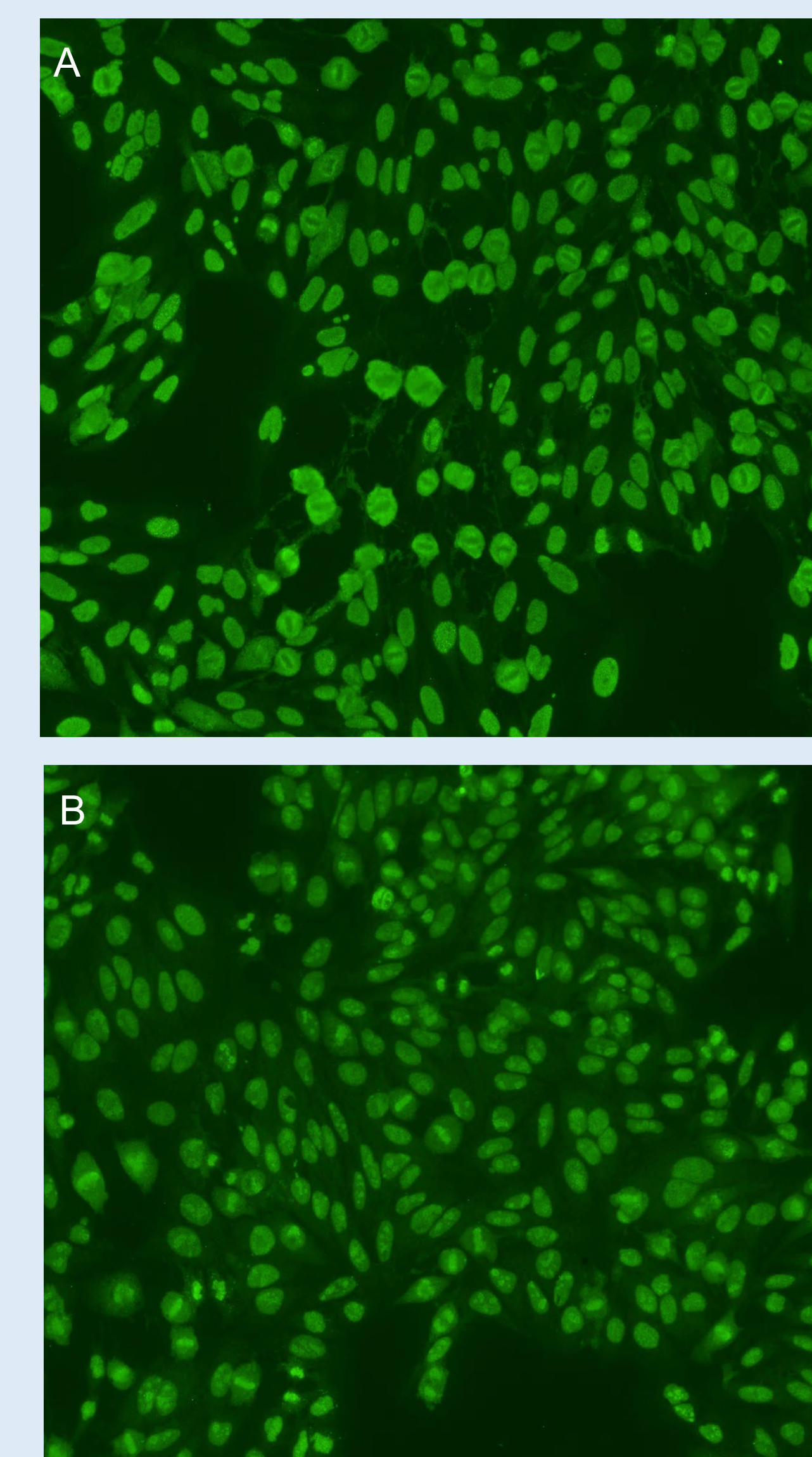

Figure 1. IIF images of ANA patterns. IIF images were evaluated with respect to # fluorescence intensity, pattern, and titer. Shown # are the most common patterns including: A. Speckled, B. Nucleolar.

Antibody	Triple Negative (n=40)	ILD n (%)	ILD RR (95% CI)	ILD p-value	MRSS >12 n (%)	MRSS >12 RR (95% CI)	MRSS >12 p-value	CK (>145) n (%)	CK (>145) RR (95% CI)	CK (>145) p-value	Digital Ulcers + n (%)	Digital Ulcers + RR (95% CI)	Digital Ulcers + p-value
Ro-52	20 (50)	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
Th/To	16 (40)	10 (63)	1.07 (0.61 - 1.77)	>0.99	2 (13)	0.5 (0.12 - 1.85)	0.44	7 (44)	1.33 (0.59 - 2.96)	0.51	7 (44)	0.87 (0.42 - 1.67)	0.75
MDA5	14 (35)	8 (57)	1.11 (0.59 - 1.88)	0.76	4 (29)	1.86 (0.57 - 5.88)	0.42	4 (29)	0.6 (0.23 - 1.40)	0.31	8 (57)	1.35 (0.68 - 2.52)	0.51
SAE1	11 (27.5)	6 (55)	1.00 (0.48 - 1.71)	>0.99	2 (18)	0.88 (0.22 - 3.10)	>0.99	3 (27)	0.68 (0.23 - 1.66)	0.7	6 (55)	1.22 (0.57 - 2.25)	0.73
Fibrillarin	10 (25)	3 (30)	0.51 (0.18 - 1.12)	0.16	2 (20)	1.00 (0.25 - 3.46)	>0.99	5 (50)	1.39 (0.58 - 2.95)	0.47	6 (60)	1.38 (0.66 - 2.52)	0.47
PM75	10 (25)	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**
Ku	9 (22.5)	7 (78)	1.46 (0.80 - 2.29)	0.26	2 (22)	1.15 (0.28 - 3.90)	>0.99	4 (44)	1.60 (0.63 - 3.29)	0.4	3 (33)	0.65 (0.22 - 1.43)	0.46
PM100	9 (22.5)	7 (78)	1.46 (0.80 - 2.29)	0.26	2 (22)	1.15 (0.28 - 3.90)	>0.99	5 (56)	1.60 (0.68 - 3.34)	0.43	6 (67)	1.59 (0.77 - 2.82)	0.26
Mi-2b	8 (20)	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

Table 2. Antibody prevalence by immunoblot. Presence of scleroderma and myositis specific antibodies were assessed by immunoblot in the triple negative cohort (n=40). Antibodies with a prevalence <20% are not shown (n=20). For each clinical outcome (interstitial lung disease (ILD), modified Rodnan skin score (MRSS), creatine kinase (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 highlighted.

Results

Antibody Prevalence (Table 2)

- Of 29 autoantibodies tested, the most prevalent were Ro-52 (50%), Th/To (40%), MDA5 (35%), SAE1 (28%), PM-75 (25%), fibrillarin (25%).

- 98% of patients had at least one positive autoantibody. 75% of patients had ≥3 antibodies and was associated with worse lung fibrosis.

- Ro-52 was associated with ILD (RR 2.67, p<0.001) and CK (RR 2.64, p<0.05).

- PM-75 was associated with digital ulcers (RR 2.18, p<0.05). Mi-2b was associated with MRSS >12 (RR 4.00, p<0.05).

- There was a high prevalence of myositis specific antibodies (MSA 32.5%) and myositis associated antibodies (MAA 30%).

Conclusions

- 14% of two well described SSc populations were characterized as ANA+ triple negative SSc.

- ANA positive patients negative for the prototypic SSc antibodies were clinically heterogeneous, demonstrated a variety of autoantibodies not routinely clinically assessed, many of which were associated with specific clinical manifestations.

- Triple negative patients demonstrated an equal prevalence of limited and diffuse cutaneous SSc, high prevalence of digital ulcers, myopathy, and ILD.

- Ro-52 was the most prevalent antibody (50%) and was associated with increased risk of ILD and elevated CK, confirming previous studies which showed association of Ro-52 with ILD and inflammatory myositis. Additional common antibodies in this subset included Th/To, MDA5, SAE1, PM-75, fibrillarin.

- Many patients had multiple autoantibodies and this was associated with more severe lung disease.

- There was a high prevalence of myositis specific antibodies.

- ANA positive triple negative SSc is a relatively common but clinically diverse entity and clinicians should recognize these patients as high risk for ILD and muscle disease.

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