Evaluation of particle-based multi-analyte technology for autoantibody detection in systemic sclerosis: concordance with conventional methods and clinical associations

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Abstract

Objective

To evaluate the clinical performance of particle-based multi-analyte technology (PMAT) for the detection of systemic sclerosis (SSc)-associated autoantibodies and to explore correlations between antibody profiles and disease manifestations.

Methods

We assessed PMAT performance in 124 SSc patients fulfilling the 2013 ACR/EULAR criteria, 205 disease controls, and 25 healthy individuals. All samples were tested for a broad autoantibody panel, including both classification and non-criteria autoantibodies. Concordance with conventional methods (chemiluminescence and ELISA) and associations with clinical features were analysed.

Results

PMAT showed excellent agreement with established techniques for anti-centromere autoantibodies (ACA) (κ=0.95) and anti-topoisomerase I (anti-Topo I) (κ=0.95), and good agreement for anti-RNA polymerase III autoantibodies (anti-RNApol III) (κ=0.78), confirming its reliability in detecting major SSc autoantibodies. The most prevalent autoantibodies were ACA (54.0%), anti-Topo I (21.0%), and anti-RNApol III (5.7%), each associated with distinct clinical phenotypes: ACA with limited cutaneous SSc and absence of interstitial lung disease (ILD); anti-Topo I with diffuse cutaneous SSc, ILD, and scleroderma renal crisis; and anti-RNApol III with digital ulcers and calcinosis. Importantly, novel associations were identified between PUF60 isoform 6 and severe ILD, and between PUF60 isoform 1 and the sine scleroderma phenotype, suggesting their potential as emerging biomarkers.

Conclusion

These findings support the diagnostic and clinical utility of comprehensive serological profiling and the integration of PMAT into routine diagnostic workflows. The identification of novel autoantibody-phenotype associations underscore the value of multiplex technologies in advancing precision medicine for SSc.

Key words

systemic sclerosis, scleroderma, antibodies, assessment biomedical technology, clinical associations

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Introduction

Systemic sclerosis (SSc) is a rare, chronic systemic autoimmune disorder characterised by excessive deposition of connective tissue components in response to injury, leading to fibrosis and vasculopathy. Although more than 90% of patients present with Raynaud's phenomenon, the disease exhibits marked clinical heterogeneity (1). Classically, SSc has been classified into two major subtypes according to the extent of skin involvement: diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc). According to the LeRoy and Medsger classification of 1988, each subtype is associated with a distinct pattern of organ involvement (2). Patients with dcSSc more frequently develop interstitial lung disease (ILD), whereas pulmonary arterial hypertension (PAH) is more prevalent in lcSSc (3). Furthermore, a third subtype known as SSc sine scleroderma (ssSSc) is defined by the presence of Raynaud's phenomenon and clinical organ involvement in the absence of skin thickening, typically associated with a milder disease course compared to lcSSc (4). Finally, very early SSc (VEDOSS) refers to patients identified at the earliest stages of the disease, who do not yet meet the 2013 ACR/EULAR classification criteria for the disease and are defined by the EUSTAR-proposed criteria comprising Raynaud's phenomenon, puffy fingers, antinuclear antibody (ANA) positivity, and SSc-specific autoantibodies and/or abnormal nailfold capillaroscopy findings (5).

The pathogenic role of autoantibodies (Abs) in SSc remains incompletely understood. However, emerging evidence suggests that specific Ab profiles may be more predictive of clinical manifestations than the extent of skin involvement (6, 7). Data from the Spanish Scleroderma Registry (RESCLE) demonstrated that the presence of anti-topoisomerase I/Scl-70 (anti-Topo I) or anti-centromere/CENP-B (ACA) was associated with 13 distinct clinical features, while skin involvement (diffuse or limited) defined another 13 (8). Similar findings were also reported by the European League Against Rheumatism (EULAR) Scleroderma Trials and Research (EUSTAR) group (9). Additionally, anti-RNA-polymerase III Abs (anti-RNApol III), have been associated with an increased risk of scleroderma renal crisis and synchronous cancer (8). These three Abs, anti-Topo I, ACA, and anti-RNApol III, are included in the 2013 American College of Rheumatology (ACR)/EULAR classification criteria for SSc (10).

Unlike other systemic autoimmune diseases such as systemic lupus erythematosus, patients with SSc typically have a monospecific autoantibody profile, with only one of the SSc-specific antibodies included in the classification criteria (11). Although some individuals lack these three major antigenic specificities, almost all patients with SSc present positive ANA, detected by indirect immunofluorescence on HEp-2 cells (IIF-HEp-2). Several additional Abs have been described in SSc, including those targeting Th/To, U3-RNP/fibrillarin, Ku, the PM/Scl complex, NOR90, PDGFR, and Ro52/TRIM21 (Ro52). While assays for detecting SSc-criteria Abs (anti-Topo I, ACA, and anti-RNApol III) are widely available, methods for identifying non-criteria SSc Abs remain limited (1). Immunoprecipitation is still considered the gold standard, but its availability is restricted in most diagnostic laboratories. Consequently, line blot and enzyme-linked immunoassays (ELISA) have emerged as reliable alternatives (12). More recently, a novel particle-based multianalyte technology (PMAT) has been developed and evaluated in preliminary studies (1, 13-15).

The main objective of the present study was to evaluate the clinical performance of PMAT for the detection of SSc-associated autoantibodies, in comparison with established laboratory techniques such as ELISA and chemiluminescence assay (CIA), and to examine the relationship between serological profiles obtained by these methods and the clinical manifestations across the spectrum of SSc.

Patients and methods

Patient characteristics and samples

Serum samples from 124 patients with SSc fulfilling the 2013 ACR/EULAR classification criteria were analysed.

All patients were followed at the Department of Autoimmune Diseases of Hospital Clínic, Barcelona and had been previously enrolled in the RESCLE. Clinical data, including cutaneous phenotype, peripheral vascular and musculoskeletal manifestations, and involvement of the gastrointestinal tract, lung, and kidneys, were reviewed from electronic medical records.

To assess specificity, 205 disease control patients with other autoimmune and non-autoimmune conditions were included: idiopathic inflammatory myopathies (IIM, n=35), autoimmune liver disease (AID, n=51), autoimmune thyroiditis (AIT, n=20), Crohn's disease (CD, n=20), infectious disease (ID, n=20), idiopathic pulmonary fibrosis/interstitial pneumonia with autoimmune features (IPF/IPAF, n=28), rheumatoid arthritis (RA, n=26), and other connective tissue disease (CTD, n=5). Moreover, 25 healthy individuals were also included as negative controls.

The study was approved by the Ethics Committee of Hospital Clínic de Barcelona (ref. HCB/2017/0947), and written informed consent was obtained from all patients and blood donors.

Immunoassays

- Indirect immunofluorescence on HEp-2 cells

Of the 124 samples, 122 were tested using IIF-HEp-2 cells (HEp-2 ANA Slide, Inova Diagnostics, San Diego, USA). Results were reported according to the International Consensus on ANA Patterns (ICAP) (16, 17). Only the AC-3 pattern was specifically reviewed to compare these results with those obtained by PMAT.

- Anti-Topo I and anti-RNApol III detection by CIA and ELISA, respectively

Prior to PMAT implementation, anti-Topo I and anti-RNApol III were routinely quantified using CIA and ELISA, respectively. The following kits were used: QUANTA Flash® Scl70 (cat no. 701328, Werfen) and QUANTA Lite® RNA Pol III (cat no. 704555, Werfen). Manufacturer-recommended cut-off values were 20 CU and 20 U/mL, respectively.

- Particle-based multi-analyte technology (PMAT) - Aptiva®

The Aptiva system employs PMAT, a fully automated technique, that enables the simultaneous detection of multiple autoantibody specificities from a single patient sample within one analytical run. The assay uses distinct populations of paramagnetic microparticles, each with a unique optical signature and coated with a specific antigen, allowing individual analyte identification. Patient samples are automatically processed and incubated with the antigen coated particles to allow binding of specific IgG autoantibodies, followed by automated washing steps and the addition of a phycoerythrin conjugated anti human IgG detection antibody. An internal control particle population is included to verify proper sample and reagent handling during each reaction.

After completion of the immunoreaction, particles are analysed using digital imaging technology. Light emitting diodes are used both to identify each particle population and to measure fluorescence intensity, which is proportional to the amount of bound autoantibody. Median fluorescence intensity values are converted into quantitative results using analyte specific calibration curves derived from manufacturer provided calibrators. Through the combination of multiplexing, digital image based detection, and standardised calibration procedures, the PMAT based Aptiva system delivers reproducible, accurate, and analytically robust measurements within a fully automated workflow.

All patient samples were tested using PMAT assays on Aptiva® instrument (Inova Diagnostics, San Diego, USA). The following autoantibodies were assessed using the Aptiva CTD Essential and CTD Comprehensive panels: ACA, anti-Topo I, Ro52, U1-RNP, anti-RNApol III, Th/To (Rpp25), Th/To (Rpp38), PM/Scl, Ku, intracellular protein bicaudal D2 (BICD2), and Fibrillarin (U3-RNP) [Research Use Only]. Additionally, exploratory markers we evaluated using early feasibility reagents: PUF60 (isoforms 1, 2 and 6), RNPC3r, RNPC3 (peptide 1-5), RuvBL1/2, and telomeric repeat binding factor 1 (TERF1) [research use only].

The prevalence of Abs was determined based on the manufacturer's recommended cut-off values. Results from the Aptiva® CTD Essential panel were expressed in *calibrated* fluorescent intensity units (FLU), while novel biomarkers were reported in *uncalibrated* median fluorescent intensity (MFI).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, v. 23 (IBM Corp., Armonk, N.Y., USA) and GraphPad Prism for Windows, v. 8.3.0 (GraphPad Software, La Jolla, California, USA). Statistical significance was set at p-values <0.05. Continuous variables were described as mean and standard deviation or median and interquartile range (IQR: 25^{th}-75^{th} percentile), depending on data distribution. Categorical variables were expressed as absolute numbers and percentages. Differences in proportions were analysed using Fisher's exact test, whereas differences in the means of continuous variables were analysed using the parametric Student's t-test or the Mann-Whitney non-parametric U-test when the data were not normally distributed. Associations between categorical variables were determined using Fisher's exact test. The relative measure of an effect was expressed as odds ratio (OR) with 95% confidence interval (CI), considering autoantibody positivity as the exposure variable.

Results

Clinical characteristics

The study cohort included 124 patients with SSc, of whom 111 (89.5%) were women and 13 (10.5%) men. All patients tested positive for ANA by IIF-HEp-2 cells. Regarding cutaneous phenotype, lcSSc was the most prevalent subtype (61/124; 49.2%), followed by ssSSc (38/124; 30.7%). dcSSc was observed in 23 patients (18.6%) and 2 patients (1.6%) were classified as having very early SSc. A detailed summary of demographic characteristics and clinical features is provided in Table I.

Correlation between PMAT and conventional methods

A very good agreement was observed

between PMAT and conventional assays for the three Abs included in the 2013 ACR/EULAR classification criteria. ACA results from PMAT were compared with the AC-3 pattern observed by IIF-HEp-2, while anti-Topo I and anti-RNApol III results from PMAT were compared with CIA and ELISA, respectively.

Regarding ACA, among the 122 patients with both IIF and PMAT results, 64/66 (97.0%) with an AC-3 pattern were also ACA-positive by PMAT, yielding a *kappa* of 0.950 (95% CI 0.773–1.000). Discordant results, between IIF and PMAT, were observed in only three cases: two patients were ACA-positive by IIF-HEp-2 but negative for PMAT (2.17 and 3.75 FLU, respectively), and one patient was ACA-positive by PMAT (18.73 FLU) without an AC-3 pattern on IIF-HEp-2 (AC-5 positive pattern).

Regarding anti-Topo I, only 2 out of 123 (1.6%) patients showed discordant results. One out of 25 anti-Topo I-positive by CIA was negative by PMAT, and 1 of 98 CIA-negative cases was positive by PMAT. This comparison also yielded a kappa of 0.950 (95% CI 0.773–1.000), indicating excellent agreement.

Finally, anti-RNApol III showed slightly lower agreement, with a kappa of 0.781 (95% CI 0.571–0.990). Among 83 patients with available anti-RNApol III results, 6 positive and 74 negative cases were concordant, while 3 patients were positive by ELISA but negative by PMAT. These 3 discordant patients had ELISA values of 28, 29 and 71 U/mL, considered low-positive. In contrast, ELISA values for the other 6 positive patients ranged from 90 to 200 U/mL, with one patient at 50 U/mL, suggesting that PMAT may have reduced sensitivity for low-titre anti-RNApol III detection.

Autoantibodies profile in SSc

The frequency of Abs in SSc patients is shown in Table II. Concerning the classification criteria Abs, anti-Topo I was detected in 26 SSc patients (21.0%), ACA in 67 (54.0%), and anti-RNApol III in 7 (5.7%). Only two patients tested positive for both anti-Topo I and ACA

Table I. Clinical characteristics of the SSc patient population.

	Patients with dcSSc (n=23)	Patients with lcSSc (n=61)	Patients with ssSSc (n=38)
Demographic characteristics			
Sex (female)	17 (73.9)	55 (90.2)	37 (97.4)
Age at disease diagnosis (years)	43 ± 12	45 ± 16	47 ± 17
Duration of the disease (years)	12.0 ± 10.6	14.7 ± 12.1	13.4 ± 12.3
Clinical manifestations			
Raynaud's phenomenon	21 (91.3)	58 (95.1)	34 (89.5)
Digital ulcers	15 (65.2)	11 (18.0)	2/37 (5.4)
Telangiectasias	19 (82.6)	44/60 (73.3)	18/37 (48.7)
Calcinosis	8 (34.8)	16/60 (26.7)	1 (2.6)
Arthritis	---	3 (4.9)	1 (2.6)
Myositis	1 (4.4)	6 (9.8)	1/36 (2.8)
Oesophageal involvement	16/22 (72.7)	41/60 (68.3)	19/37 (51.4)
Interstitial lung disease	18 (78.3)	22 (36.1)	7 (18.4)
Severe interstitial lung disease	9 (39.1)	3 (4.9)	---
Pulmonary arterial hypertension	1/22 (4.5)	12/60 (20.0)	6 (20.7)
Scleroderma renal crisis	2 (8.7)	---	0/37 (0.0)
Diastolic dysfunction	5 (21.7)	17/59 (28.8)	6 (20.7)
Pericarditis	4 (17.4)	3/60 (5.0)	---
Conduction alterations	3 (13.0)	3/60 (5.0)	4 (10.5)
Neoplasia	3 (13.0)	7 (11.5)	1 (2.6)

Quantitative variables are expressed as mean ± SD qualitative variables are expressed as number (percentage).
dsSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ssSSc: sine scleroderma; SD: standard deviation.

Table II. Frequency of classification and non-classification criteria autoantibodies.

	Patients with dcSSc (n=23)	Patients with lcSSc (n=61)	Patients with ssSSc (n=38)
Classification criteria Abs			
Anti-Topo I	14 (60.9)	8 (13.1)	4 (10.5)
ACA	2 (8.7)	36 (59.0)	27 (71.1)
Anti-RNApol III	4 (17.4)	3 (4.9)	---
Negative for classification criteria Abs	4 (17.4)	14 (23.0)	8 (21.1)
Non classification criteria Abs			
Ro52/TRIM21	5 (21.7)	6 (9.8)	11 (29.0)
U1-RNP	2 (8.7)	3 (4.9)	3 (7.9)
PM/Scl	2 (8.7)	3 (4.9)	1 (2.6)
Ku	2 (8.7)	---	2 (5.3)
Th/To (Rpp25)	---	---	1 (2.6)
Th/To (Rpp38)	1 (4.3)	1 (1.6)	1 (2.6)
U3-RNP/Fibrillarin	---	1 (1.6)	1 (2.6)
BICD2	---	---	1 (2.6)
Early feasibility Abs			
PUF60 isoform 1	1 (4.3)	3 (4.9)	6 (15.8)
PUF60 isoform 2	---	2 (3.3)	2 (5.3)
PUF60 isoform 6	3 (13.0)	1 (1.6)	1 (2.6)
RNPC3r	---	---	---
RNPC3 P1	---	---	---
RNPC3 P2	---	---	---
RNPC3 P3	---	---	---
RNPC3 P4	1 (4.3)	1 (1.6)	1 (2.6)
RNPC3 P5	---	---	---
RuvBL1	1 (4.3)	2 (3.3)	---
RuvBL2	1 (4.3)	1 (13.1)	---
TERF1	---	1 (13.1)	1 (2.6)

Quantitative variables are expressed as mean ±SD qualitative variables are expressed as number (percentage).
dsSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc; ssSSc: sine scleroderma; SD: standard deviation; Abs: autoantibodies; anti-Topo I: anti-topoisomerase I autoantibodies; ACA: anti-centromere autoantibodies; anti-RNApol III: anti-RNA polymerase III autoantibodies.

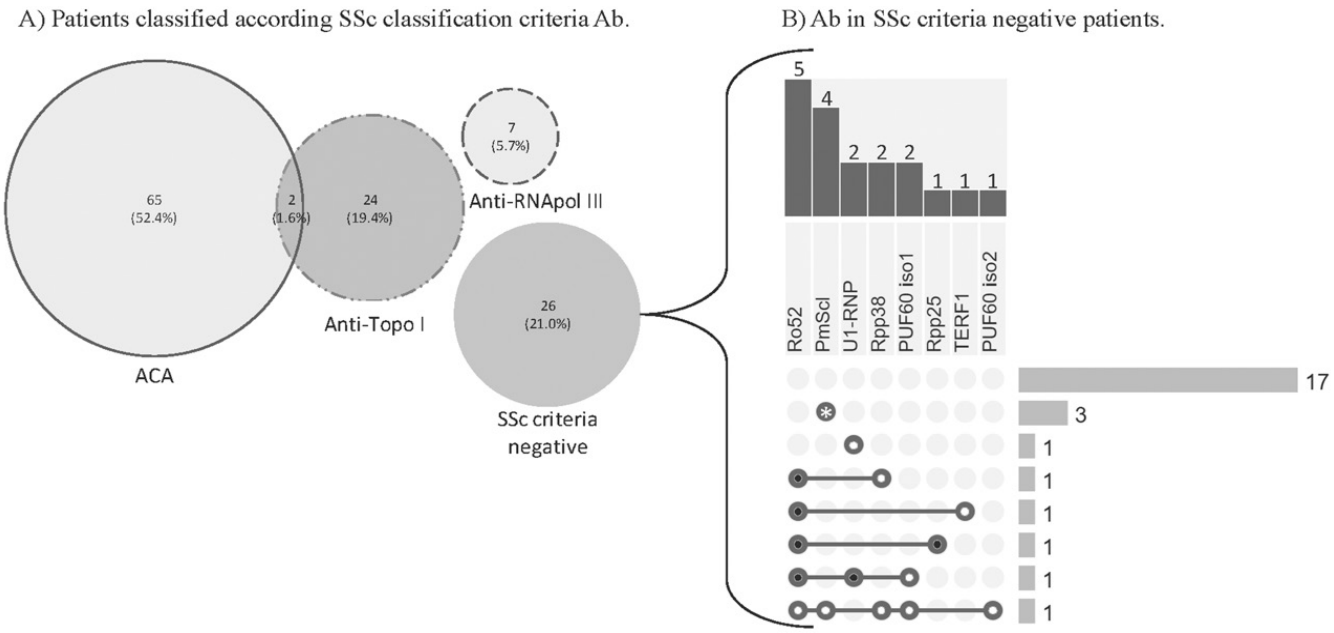

Fig. 1. Prevalence of classification and non-classification criteria autoantibodies.
A: Venn diagram showing the overlap between the SSc-classification criteria Abs (anti-Topo I, ACA or anti-RNApol III) among SSc patients (n=124).
B: Upset plot displaying the frequency and intersection between SSc-non classification criteria Abs among patients that are negative for SSc classification criteria Abs (n=29) (only markers with at least one positivity are shown). White dots show positivity <5x cut-off and black dots represent positivity ≥5x cut-off.
*1 out of 3 patients had low PM/Scl titres (<5x cut-off).

simultaneously (Fig. 1a). Thus, the remaining 26/124 (21.0%) were negative for all three classification criteria Abs. Of these, 17/26 (65.4%) were also negative for all other tested Abs. The remaining 9 patients (34.6%) showed either single or multiple positivity for other Abs, including anti-PM/Scl, anti-U1-RNP, anti-Th/To (Rpp25 and Rpp38), anti-TERF1, anti-PUF60 (iso1 and iso2), and anti-Ro52 Abs (Fig. 1b).

Associations between Abs with clinical features in SSc

As expected, ACA, anti-Topo I and anti-RNApol III were associated with distinct cutaneous phenotype, and both anti-Topo I and anti-RNApol III were associated with the presence of ILD. Additionally, anti-RNApol III was associated with calcinosis, and anti-Topo I exhibited a markedly increased risk of scleroderma renal crisis. Moreover, both anti-Topo I and anti-RNApol III were associated with digital ulcers, whereas ACA was associated with the absence of ILD and digital ulcers. Notably, anti-PUF60 isoform 6 Ab was associated with diffuse cutaneous phenotype and severe ILD, while anti-PUF60 isoform 1 was associated with ssSSc phenotype. Interestingly, Abs against Rpp38 and RuvBL1 were more frequently found in patients with ILD, although these associations did not reach statistical significance. No significant associations were found between any of the studied Abs and neoplasia, cardiac arrhythmias or conduction disturbances, pericarditis or diastolic dysfunction, myositis, arthritis, pulmonary hypertension or telangiectasias. Additionally, no significant associations were observed within the only ANA-positive group. Significant associations between clinical features and Abs are shown in Table III.

Prevalence of Abs and associations with SSc, disease controls and healthy individuals

A distinct autoantibody profile was observed when compared SSc patients to disease controls (n=205) and healthy individuals (n=25) (Supplementary Table S1, Suppl. Fig. S1). ACA was the most prevalent (54.0%), followed by anti-Topo I (21.0%) and anti-Ro52 antibodies (17.7%), all showing highly significant associations with SSc ($p<0.001$, $p<0.001$, and $p=0.003$, respectively). Additional antibodies such as anti-RNApol III (5.6%), anti-PM/Scl (4.8%), and PUF60 isoform 1 (8.1%) also demonstrated statistically significant enrichment in the SSc group compared to controls. In contrast, Abs targeting U1-RNP, Ku, RNPC3 and RuvBL1/2 did not show statistically significant differences among the groups.

Analysis of control groups showed that ACA, anti-Topo I, anti-RNApol III, Pm/Scl, fibrillarin, BICD2, all RNPC3-derived peptides, RPP25, RPP38, RUVBL1, RUVBL2, and TERF1 exhibited 100 % specificity, with no reactivity detected in either healthy individuals or disease controls whereas Ku, U1-RNP, Ro52, and PUF60 isoforms displayed very high specificity, ranging from 99.6% to 93%, due to low-frequency positivity in autoimmune controls (Suppl. Table S1).

Discussion

In this study we evaluated the prevalence and clinical relevance of SSc-associated autoantibodies using three complementary immunoassay platforms: ELISA, CIA, and PMAT. Multiparametric platforms such as PMAT enable simultaneous measurement of multiple specificities, offering advan-

Table III. Significant associations (odds ratios) between clinical manifestations and autoantibodies.

		SSc-criteria autoantibodies			Non-criteria SSc autoantibodies							Novel autoantibodies								
	n	ACA	Anti-Topo I	Anti-RNApol III	Ro52	U1-RNP	PM/Scl	Ku	Th/To (Rpp25)	Th/To (Rpp38)	U3-RNP (Fibrillarin)	BICD2	PUF60 iso1	PUF60 iso2	PUF60 iso6	RNPC3 P4	RuvBL1	RuvBL2	TERF1	Only ANA (IIF-Hep2) positive
	n	67	26	7	22	9	6	4	1	3	2	1	10	4	5	3	3	2	2	17
Cutaneous phenotype																				
Diffuse SSc	23	0.053	11.537	6.877			5.105								7.425					
Limited SSc	61		0.377		0.320															
Sine scleroderma	38	2.823			2.778								3.844							
Clinical manifestations																				
Raynaud phenomenon	115									0.031										
Digital ulcers	28	0.316	2.743	10.109																
Telangiectasias	82																			
Calcinosis	25			30.632																
Arthritis	4																			
Myositis	8																			
Esophageal involvement	77																			
Interstitial lung disease	47	0.078	6.786	28.701			5.488													
Severe interstitial lung disease	12	0.027	6.853	9.000											7.267					
Scleroderma renal crisis	2		19.898																	
Diastolic dysfunction	28																			
Pulmonary arterial hypertension	19																			
Pericarditis	7																			
Conduction alterations	10																			
Neoplasia	11																			

Only odds ratios of significant associations are shown. Negative (protective) associations are highlighted in grey and positive (risk) associations in black.
Non significant associations between serology and clinical manifestation are shown as empty (white) squares.
ACA: anti-centromere autoantibodies; anti-Topo I: anti-topoisomerase I autoantibodies; anti-RNApol III: anti-RNA polymerase III autoantibodies; IIF-Hep2: indirect immunofluorescence on HEp-2 cells; SSc: systemic sclerosis.

tages in throughput and the comprehensiveness of serological profiling that can enhance diagnostic sensitivity and support clinical stratification (18). Based on these considerations, we assessed the performance of PMAT and compared its results with established techniques.

Our comparative analysis demonstrates that PMAT provides high concordance with established methods for the major SSc-defining antibodies such as ACA and anti-Topo I (kappa of 0.95 for both), and good concordance for anti-RNApol III (kappa of 0.78), thereby supporting its potential utility in routine diagnostic workflows.

Consistent with prior literature, ACA, anti-Topo I and anti-RNApol III were the most prevalent antibodies and retained their well-established phenotype associations. Beyond these canonical markers, PMAT enabled the detection of several non-criteria antibodies and exploratory specificities. Notably, we identified associations between PUF60 isoform 6 and severe ILD and between PUF60 isoform 1 and the sine-scleroderma phenotype. To our knowledge these associations have not been previously reported and, if validated, could expand the repertoire of biomarkers useful for phenotypic refinement and risk stratification.

Alkema *et al*. compared multiple commercial platforms for SSc-specific (ACA, anti-Topo I, anti-RNApol III [RP11/155]) and SSc-associated Abs (Fibrillarin, Th/To, PM/Scl75, PM/Scl100, RNP68/A/C, Ku, NOR90, and PDGFR) in 347 SSc patients (12). High agreement was reported for SSc-specific Abs (Cohen's kappa 0.53-0.97), while lower concordance was observed for PM/Scl, Ku, fibrillarin, and Th/To.

The literature on uncommon SSc antibodies is heterogeneous. Some studies report robust phenotype associations for markers such as fibrillarin, PM/Scl, and RuvBL1/2, whereas others show limited reproducibility across platforms and cohorts (19,20). For example, anti-RNPC-3 (U11/U12) has been linked to ILD and, in earlier reports, to cancer-associated scleroderma, although subsequent studies have produced inconsistent results (21-23). Similarly, antibodies such as BICD2, RuvBL1/2 and TERF1 have been described as highly specific for SSc and associated with particular clinical patterns in independent cohorts (24-26). Our findings align with these reports in suggesting that an expanded serological panel can reveal clinically meaningful associations, but they also reinforce the need for replication and functional characterisation.

PMAT's multiplex format allows parallel assessment of a broad antibody

repertoire with minimal sample volume and reduced hands-on time, which is particularly advantageous when screening for both classification and non-criteria specificities. The incorporation of multiplex platforms such as PMAT, complemented by established techniques (ELISA and CIA), demonstrates promising technical viability for expanding the repertoire of autoantibodies detectable in SSc and offers clear avenues for clinical translation. Analytically, PMAT's capacity to measure multiple specificities in parallel enhances throughput and enables more comprehensive serological profiling while maintaining high specificity for major SSc antibodies. However, observed discordances at low titres (notably for anti-RNApol III) highlight the need for optimisation of limits of detection, inter-assay calibration, and rigorous quality-control procedures to ensure consistent sensitivity across platforms.

From a clinical standpoint, a validated, standardised expanded antibody panel could refine phenotypic stratification, facilitate earlier identification of patients at risk for organ complications, and inform risk-adapted monitoring and therapeutic decision-making. To realise these benefits, multicentre and longitudinal validation studies are required to assess reproducibility, prognostic value and temporal dynamics of novel specificities. Economic evaluations should determine cost-effectiveness in routine care and harmonisation efforts (encompassing assay standardisation, reference materials, and regulatory and laboratory-quality frameworks) are essential to translate multiplex serology into robust, clinically actionable biomarkers for precision management of SSc.

Our study has several limitations. The sample size, while representative, may not capture the full spectrum of rare Abs or less common clinical manifestations and comorbidities. The cross-sectional design limits causal inference between Abs presence and disease progression. Additionally, while PMAT demonstrated excellent overall agreement with conventional methods, discrepancies in low-positive results, particularly for anti-RNApol III, suggest areas where further validation could help optimise performance.

Nevertheless, the study has notable strengths. It includes a well-characterised cohort of SSc patients with detailed clinical phenotyping and two control groups, enhancing the specificity of the findings. The use of different immunoassay techniques allow for robust comparison and validation of Abs detection. Importantly, the inclusion of novel and research-use-only Abs provides insights into emerging biomarkers that may refine future classification and prognostic models.

Conclusion

Our findings reinforce the strong diagnostic, clinical, and prognostic value of ACA, anti-Topo I, and anti-RNApol III in SSc, with each autoantibody showing distinct associations with specific disease phenotypes. Combining advanced multiplex technologies, like PMAT, with expanded biomarker panels, including PUF60, holds promise for improving early diagnosis, prognostic assessment, and personalised management in systemic sclerosis.

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