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Clinical Phenotypes of Patients With Systemic Sclerosis With Distinct Molecular Signatures in Skin

Monica Yang,[1] Vivien Goh,[2] Jungwha Lee,[2] Monica Espinoza,[3] Yiwei Yuan,[3] Mary Carns,[2] Kathleen Aren,[2] Lorinda Chung,[4] Dinesh Khanna,[5] Zsuzsanna H. McMahan,[6] Rishi Agrawal,[2] Lauren Beussink Nelson,[2] Sanjiv J. Shah,[2] Michael L. Whitfield,[3] and Monique Hinchcliff[7]

Objective. Systemic sclerosis (SSc) patients are classified according to degree of skin fibrosis (limited and diffuse cutaneous [lc and dc]) and serum autoantibodies. We undertook the present multicenter study to determine whether intrinsic subset (IS) classification based upon skin gene expression yields additional valuable clinical information.

Methods. SSc patients and healthy participants (HPs) were classified into Normal-like, Limited, Fibroproliferative, and Inflammatory ISs using a previously trained classifier. Clinical data were obtained (serum autoantibodies, pulmonary function testing, modified Rodnan skin thickness scores [mRSS], and high-resolution chest computed tomography [HRCT]). Statistical analyses were performed to compare patients classified by IS, traditional cutaneous classification, and serum autoantibodies.

Results. A total of 223 participants (165 SSc [115 dcSSc and 50 lcSSc] and 58 HPs) were classified. Inflammatory IS patients had higher mRSS (22.1 ± 9.9; $P < 0.001$) than other ISs and dcSSc patients (19.4 ± 9.4; $P = 0.05$) despite similar disease duration (median [interquartile range] months 14.9 [19.9] vs. 18.4 [31.6]; $P = 0.48$). In multivariable modeling, no significant association between mRSS and RNA polymerase III ($P = 0.07$) or anti–topoisomerase I (Scl-70) ($P = 0.09$) was found. Radiographic interstitial lung disease (ILD) was more prevalent in Fibroproliferative IS compared with other ISs (91%; $P = 0.04$) with similar prevalence between lcSSc and dcSSc (67% vs. 76%; $P = 0.73$). Positive Scl-70 antibody was the strongest ILD predictor ($P < 0.001$). Interestingly, all lcSSc/Fibroproliferative patients demonstrated radiographic ILD.

Conclusions. Classification by IS identifies patients with distinct clinical phenotypes versus traditional cutaneous or autoantibody classification. IS classification identifies subgroups of SSc patients with more radiographic ILD (Fibroproliferative), higher mRSS (Inflammatory), and milder phenotype (Normal-like) and may provide additional clinically useful information to current SSc classification systems.

INTRODUCTION

Systemic sclerosis (SSc; scleroderma) is a phenotypically diverse collagen vascular disease whose clinical hallmark is fibrosis of the skin and internal organs. Autoimmunity with autoantibody production and vascular dysfunction are thought to occur earlier in the disease course, followed by progressive fibrosis and organ dysfunction in a subset of patients. Although 2 SSc subtypes (limited cutaneous [lcSSc] and diffuse cutaneous [dcSSc]) have been clinically identified based on the pattern of skin fibrosis as defined by Leroy et al (1), this traditional cutaneous classification system does not reliably predict disease severity, progression, and therapeutic response (2). Furthermore, in light of uniformly negative recent trial results in SSc cutaneous disease (3,4), there is an unmet need for identifying and enrolling subgroups of SSc patients that may enhance treatment efficacy. In 2008, a new SSc classification

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[1]Monica Yang, MD: University of California San Francisco, San Francisco; [2]Vivien Goh, MD, Jungwha Lee, MS, PhD, Mary Carns, MS, Kathleen Aren, MA, MPH, Rishi Agrawal, MD, Lauren Beussink Nelson, RDCS, MPH, Sanjiv J. Shah, MD: Northwestern University Feinberg School of Medicine, Chicago, Illinois; [3]Monica Espinoza, BS, Yiwei Yuan, BS, Michael L. Whitfield, PhD: Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire; [4]Lorinda Chung, MD, MS: Stanford University, Palo Alto, California; [5]Dinesh Khanna, MD, MS: University of Michigan, Ann Arbor; [6]Zsuzsanna H. McMahan, MD, MHS: Johns Hopkins University, Baltimore, Maryland; [7]Monique Hinchcliff, MD, MS: Yale School of Medicine, New Haven, Connecticut.

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Address correspondence via email to Monica Yang, MD, at Monica.yang@ucsf.edu.

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SIGNIFICANCE & INNOVATIONS

- The intrinsic subset skin gene expression classification system is a newer systemic sclerosis (SSc) classification that may help identify patients with radiographic interstitial lung disease and more skin fibrosis compared with the traditional cutaneous classification (limited cutaneous [lc] and diffuse cutaneous) and serum autoantibody status.
- Significance lies in the ability to enrich SSc clinical trials for patients with similar molecular programs underlying skin disease who may respond to targeted treatment.
- Innovation lies in the potential to identify SSc subgroups that may not otherwise be identified by the traditional cutaneous classification and serum autoantibodies, including patients with lcSSc who are at increased risk for interstitial lung disease.

system termed intrinsic subset (IS) classification based upon skin gene expression was identified (5). The current study makes use of high-quality and comprehensive clinical data to determine the clinical phenotype of SSc patients classified according to IS and how this novel classification system compares and complements traditional cutaneous and autoantibody classification.

Four ISs—Normal-like, Limited, Fibroproliferative, and Inflammatory—have been defined (5). Since their discovery, these ISs have been validated in several additional SSc cohorts and found to be expressed in skin and esophageal tissues (6–10). Furthermore, the IS model has been increasingly utilized in post hoc analysis of clinical studies and trial data to understand the presence/absence of skin disease improvement as assessed by the modified Rodnan skin thickness score (mRSS) (11–13). However, although the gene expression ISs are distinct from traditional cutaneous classification, the clinical phenotype and significance of each individual IS are not well characterized, and the clinical utility of IS classification is not established.

The study objective was to define the clinical characteristics and markers of disease severity in SSc patients classified by IS and examine the additive value of IS classification to existing systems, including traditional cutaneous and autoantibody classification. Demographic and clinical characteristics (e.g., laboratory, mRSS, pulmonary function tests [PFTs], high-resolution chest computed tomography [HRCT], echocardiogram [echo]) and patient-reported outcome instrument data were used. We hypothesized IS classification would identify distinct groups of SSc patients with similar clinical phenotypes and provide more granular information to the existing classification systems with regards to severity of skin and lung disease.

PATIENTS AND METHODS

Study population. This study reports the baseline findings of a large, multicenter, prospective cohort study that was approved by the Institutional Review Board at Northwestern University (STU00004428), University of Michigan (UM00084385), Stanford University (IRB00026772), and Johns Hopkins University (NA_00087035). Study participants provided written informed consent. Participant-level data were prospectively obtained for SSc patients and healthy participants (HPs). Paired skin biopsies of the nondominant forearm were performed: one for histology and the other for DNA microarray analyses to measure gene expression. All SSc patients fulfilled the American College of Rheumatology/EULAR 2013 classification criteria (14).

DNA microarray preparation. RNA was prepared from skin biopsies as previously reported (5,10). Tissue homogenization was performed using Qiagen TissueLyserII. RNA purification was carried out in QIAcube with Qiagen's RNeasy Mini Kit, and the Agilent2100 Bioanalyzer assessed RNA integrity. Samples had RNA integrity numbers >7. RNA concentration was measured with Thermo Scientific NanoDrop2000 Spectrophotometer, and 200 ng total RNA was amplified and labeled with Agilent Quick-Amp Labeling Kits. Cy3-labeled sample and Cy5-labeled Universal Human Reference RNA (Stratagene) were cohybridized to Agilent Human Genome (4 × 44K) Microarrays (G4112F). Data were $\log_2$ Lowess normalized and filtered for probes with intensity 2-fold or greater over local background in Cy3 or Cy5 channels. Data were multiplied by −1 to convert to Log_2(Cy3/Cy5) ratios. Probes with >20% missing data were excluded. Gene expression data are available on Gene Expression Omnibus (GSE59787).

Determination of ISs. ISs were assigned using a previously trained Glmnet machine learning classifier (15). Briefly, the training set consisted of 3 independent skin gene expression data sets, each processed separately using GenePattern. Missing values were imputed using K-Nearest Neighbors, the CollapseDataset module was run using median collapse mode, and genes were median-centered. Data sets were merged using only genes present in all 3 sets. Glmnet along with random forest, KernSmooth, and caret packages implemented in R were used to train supervised classifiers. Ten times, 3-fold cross-validation was used to train the model and assess robustness. The test set consisted of 3 additional independent SSc skin gene expression data sets. As previously published, the average classification accuracy of the single sample Glmnet machine learning classifier utilized was 87.1% compared with IS classification based upon unsupervised clustering algorithms of paired samples (15).

Patient-level data. Clinical information including demographic, laboratory, PFT, chest HRCT, echo, and patient-reported outcome instruments were collected within 3 months of the baseline skin biopsy used for gene expression analyses. Clinical characteristics included SSc subtype (lcSSc vs. dcSSc), SSc disease duration (defined as months since the first non-Raynaud

phenomenon event), current immunosuppression use, and skin biopsy date. Laboratory data included antinuclear antibody (ANA) status and measurement of SSc-specific antibodies, including anticentromere (ACA), anti-topoisomerase I (Scl-70), and anti-RNA polymerase III (RNA Pol III) (both Specialty Laboratories) and markers of inflammation including platelet count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) level (performed at the laboratory at each institution).

PFT values of interest included force vital capacity percent predicted (FVC%) and diffusing capacity of carbon monoxide percent predicted (DLCO%). Comprehensive clinical echo examinations with Doppler were obtained for patients at Northwestern University and scored by 1 research echocardiographer (LBN) according to standardized research protocols. All echo measurements were overread by a board-certified cardiologist (SJS). A designated chest radiologist with interstitial lung disease (ILD) expertise (RA) scored HRCT examinations for the degree of ground glass opacification (GGO) and fibrosis for each lung lobe to generate a total lung disease score (TLS), as previously described (16,17). Presence or absence of ILD was also determined by RA based upon the presence of morphologic features matching a recognized pattern of disease (i.e., nonspecific interstitial pneumonia, usual interstitial pneumonia) A second chest radiologist scored and classified a subset of studies for validation (18). Both assessors were blinded to clinical and laboratory data. For subjects who underwent testing at an outside institution, HRCT and echo digital images were obtained and analyzed by the radiologist (RA) and/or echocardiographer (LBN) when available.

Three patient-reported outcome questionnaires that have been validated in SSc were administered at the baseline visit. These included the Patient-Reported Outcomes Measurement Information System 29-Item General Health Profile (PROMIS-29), Functional Assessment of Chronic Illness Therapy Dyspnea (FACIT-Dyspnea), and Saint George's Respiratory Questionnaire (SGRQ) (19,20).

Statistical analysis. Descriptive statistics were calculated, and cross-sectional analyses were performed. Categorical measures were summarized using counts and percentages, whereas continuous measures were summarized by mean and SD or median and interquartile range (IQR). Student's *t*-test, analysis of variance, Wilcoxon/Mann-Whitney test or Kruskal-Wallis test (continuous variables), and chi-square test or Fisher's exact test (categorical variables) were used as appropriate. The significance level was defined as P less than 0.05. The data were analyzed using SAS, version 9.4.

Multiple linear regression models were used to evaluate continuous outcomes (mRSS, TLS, FVC%, DLCO%) individually, and exact logistic regression models were used to evaluate binary outcome (radiographic ILD presence) among ISs. Within the models, covariates included disease duration (<36 vs. ≥36 months), SSc subtype (dcSSc vs. lcSSc), SSc antibodies (ACA, Scl-70, and RNA Pol III), smoking status (current/past vs. never), and current immunosuppression (yes vs. no).

RESULTS

SSc cohort and IS classification. The study cohort consisted of 58 HPs and 165 SSc patients, including 50 (30%) with lcSSc and 115 (70%) with dcSSc. The mean ± SD age of SSc patients and HPs was 50 ± 11 years and 42 ± 13 years, respectively; $P = 0.26$. Most participants were female (83%) and White (77%). The median (IQR) SSc disease duration was 23.0 (46.3) months for the total group and 49.1 (91.5) months for lcSSc and 18.4 (31.7) months for dcSSc patients. The mean ± SD mRSS was 15.2 ± 10.2 for the total group and 5.6 ± 2.9 for lcSSc and 19.4 ± 9.4 for dcSSc patients. Distribution of serum antibodies included 10% ACA, 27% Scl-70, and 30% RNA Pol III positive. Demographic data by SSc clinical subtype and IS classification are shown in Table 1.

ISs were assigned using a previously trained IS classifier (15). A dendrogram depicting the distribution of patients classified according to IS and pattern of skin gene expression is shown in Supplementary Figure S1A, available on the *Arthritis Care & Research* website at https://onlinelibrary.wiley.com/doi/10.1002/acr.24998. The gene signature denoting the Inflammatory IS totaled 217 genes and consisted of signaling molecules such as interleukin-17 (IL-17), IL-3, CCR7, CCL2, and STAT1. Pathway analysis database accessed through G:Profiler revealed the top upregulated molecular pathways included leukocyte activation, regulation of T cell activation, granulocyte activation, and chemokine signaling pathways. The Fibroproliferative IS gene signature consisted of 741 genes including CD69, RTF, CCDC53, PIK3C3, and pathway analysis identified signatures related to extracellular matrix formation, collagen deposition, and cell cycle processes. The Normal-like IS signature consisted of 128 genes such as RPL37, KLF14, and TMEM256, and pathway analysis showed an upregulation in organic compound processes, lipid biosynthesis, and lipid metabolic processes (Supplementary Figure S1B, available on the *Arthritis Care & Research* website at https://onlinelibrary.wiley.com/doi/10.1002/acr.24998). Only 2 patients in the cohort were classified as Limited IS, likely because of intentional recruitment of patients with active skin disease, and thus there were insufficient data to characterize this subset further.

Of the 58 HPs, 54 (93%) were classified as Normal-like and 4 (7%) were classified as Fibroproliferative. Of the 165 SSc patients, 58 belonged to the Normal-like, 2 to the Limited, 72 to the Inflammatory, and 33 to the Fibroproliferative IS. Patients with dcSSc were classified in the Inflammatory (56%), Normal-like (23%), and Fibroproliferative (21%) ISs, whereas lcSSc patients belonged to the Normal-like (64%), Fibroproliferative (18%), Inflammatory (14%), and Limited (4%) ISs.

Table 1. Clinical characteristics for study participants by disease state and systemic sclerosis subtype*

Clinical characteristic	HPs (n = 58)	All SSc (n = 165)	lcSSc (n = 50)	dcSSc (n = 115)	*P* (HP vs. SSc)	*P* (dcSSc vs. lcSSc)
Age, mean ± SD years	42 ± 13	50 ± 11	49 ± 12	51 ± 11	<0.001†	0.26†
Sex, female, n (%)	42 (72)	137 (83)	44 (88)	93 (81)	0.08‡	0.26‡
Race, White, n (%)	47 (81)	125 (77)	43 (86)	82 (71)	0.41‡	0.04‡
mRSS, mean ± SD		15.2 ± 10.2	5.6 ± 2.9	19.4 ± 9.4		<0.001†
SSc disease duration, median (IQR) months		23 (46)	49 [92]	18 (32)		<0.001§
ANA positive, n (%)		153 (93)	47 (94)	106 (92)		>0.999‡
ANA pattern, n (%)						<0.001‡
Centromere		14 (10)	11 (27)	3 (3)		
Speckled		57 (39)	13 (32)	44 (46)		
Homogenous		40 (27)	13 (32)	27 (28)		
Nucleolar		25 (17)	4 (10)	21 (22)		
Autoantibodies, n (%)						
ACA		16 (10)	13 (26)	3 (3)		<0.001‡
Scl-70		44 (27)	10 (20)	34 (30)		0.25‡
RNA Pol III		50 (30)	2 (4)	48 (43)		<0.001‡
Platelet count (× 10^9/liter), median (IQR)		297 (96)	278 (93)	307 (92)		0.004§
ESR (mm/hour), median (IQR)		18 (32)	13 (24)	20 (32)		0.63§
CRP (mg/liter), median (IQR)		0.50 (0.55)	0.25 (0.35)	0.60 (0.65)		0.002§

* ACA = anticentromere; ANA = antinuclear antibody; CRP = C-reactive protein; dcSSc = diffuse cutaneous SSc; ESR = erythrocyte sedimentation rate; HP = healthy participant; IQR = interquartile range; lcSSc = limited cutaneous SSc; mRSS = modified Rodnan skin thickness score; RNA Pol III = RNA polymerase III; Scl-70 = anti-topoisomerase I; SSc = systemic sclerosis.
† Student's *t*-test.
‡ Pearson's chi-square test.
§ Wilcoxon rank–sum test.

Demographic and serologic findings. Demographic and serologic differences were observed when classifying SSc patients by IS compared with traditional cutaneous classification (Table 2). Mean age and sex among ISs were similar. There was a trend toward a higher percentage of White patients in the Normal-like IS and a higher percentage of Black patients in the Fibroproliferative IS, but the results were not significant ($P = 0.22$). Disease duration differed among ISs, with Inflammatory IS having the shortest disease duration at median 15 months (IQR 20 months) followed by Fibroproliferative IS median 21 months (IQR 35 months) Limited IS had the longest at median 121 months (IQR 8 months) followed by Normal-like median 45 months (IQR 84 months) ($P < 0.001$). Similar findings were seen within traditional cutaneous classification, with dcSSc having shorter disease duration than lcSSc patients (18 [32] vs. 49 [92] months; $P < 0.001$). At the time of skin biopsy, 24 of 165 (14.6%) SSc patients were taking immunosuppression, primarily mycophenolate mofetil (88%), followed by methotrexate (8%) and azathioprine (4%).

SSc-associated antibodies differed between ISs and were distinct from traditional cutaneous classification. ACA antibodies were more common in the Normal-like IS, whereas Scl-70 and RNA Pol III antibodies were more common in Fibroproliferative and Inflammatory IS patients, respectively ($P < 0.001$). lcSSc patients had more ACA positivity, and dcSSc patients had more RNA Pol III positivity ($P < 0.001$); however, there was no difference in Scl-70 positivity between lcSSc and dcSSc patients ($P = 0.29$). Platelet count was elevated in the Inflammatory IS compared with other ISs ($P < 0.001$). CRP levels demonstrated a similar trend ($P = 0.20$), whereas ESR was similar between ISs ($P = 0.82$). Traditionally classified patients demonstrated a similar inflammatory marker pattern, with significantly elevated platelet count ($P = 0.004$) and CRP ($P = 0.002$) in dcSSc compared with lcSSc but no difference in ESR between subtypes ($P = 0.63$).

Skin, pulmonary, and cardiac characteristics. Skin score differed significantly among ISs, with Inflammatory IS having significantly higher mRSS (22.1 ± 9.9; $P < 0.001$) followed by Fibroproliferative (12.3 ± 7.1) and then Normal-like (8.6 ± 5.9) ISs (Table 3). The Inflammatory IS had higher mRSS than the dcSSc subgroup (22.1 ± 9.9 vs. 19.4 ± 9.4; $P = 0.05$) (Figure 1A). Within the dcSSc subgroup, when patients were further stratified by IS, the Inflammatory IS also demonstrated higher mRSS compared with the Fibroproliferative and Normal-like ISs (23.8 ± 8.8 vs. 15 ± 6.2 vs. 12.2 ± 6.3; $P < 0.001$) (Table 4). mRSSs among lcSSc patients stratified by IS were similar.

Utilizing multivariable modeling of mRSS, Inflammatory IS continued to have significantly higher mRSS (adjusted mean 18.1, 95% confidence interval [95% CI] 15.2–21.1) compared with Fibroproliferative (11.3, 8.0–14.6) and Normal-like (10.0, 6.9–13.1) ISs, with IS and cutaneous classification having the most significant effects in the model (both $P < 0.001$) (Table 5). RNA Pol III positivity, Scl-70 positivity, and disease duration were not significantly associated with increased mRSS ($P = 0.07$, $P = 0.09$, and $P = 0.70$, respectively). Black race also was not associated with increased mRSS ($P = 0.41$).

Table 2. Demographic and clinical characteristics of SSc patients by IS*

Clinical characteristic	IS classification				
	Normal-like (n = 58)	Limited (n = 2)	Inflammatory (n = 72)	Fibroproliferative (n = 33)	*P*
Age, mean ± SD years	49.5 ± 11.8	56.5 ± 2.1	52.7 ± 9.8	47.3 ± 12.9	0.09†
Sex, female, n (%)	51 (88)	2 (100)	56 (78)	28 (85)	0.45‡
Race, n (%)					0.22‡
White	47 (81)	2 (100)	5 (72)	24 (73)	
Black	6 (10)	0 (0)	7 (10)	8 (24)	
Asian	2 (4)	0 (0)	3 (4)	0 (0)	
Hispanic	1 (2)	0 (0)	9 (13)	1 (3)	
SSc subtype, n (%)					<0.001‡
lcSSc	32 (64)	2 (4)	7 (14)	9 (18)	
dcSSc	26 (23)	0 (0)	65 (57)	24 (21)	
SSc disease duration, median (IQR) months	45 (84)	121 (8)	15 (20)	21 (35)	<0.001†
ANA, positive, n (%)	54 (93)	2 (100)	68 (94)	29 (88)	0.54‡
ANA pattern, n (%)					0.10‡
Centromere	9 (19)	1 (0)	3 (5)	2 (7)	
Speckled	20 (42)	2 (50)	28 (48)	8 (29)	
Homogenous	15 (31)	2 (50)	14 (24)	10 (37)	
Nucleolar	4 (8)	0 (0)	14 (24)	7 (26)	
Autoantibodies, n (%)					
ACA	11 (19)	0 (0)	3 (4)	2 (6)	0.04‡
Scl-70	15 (27)	0 (0)	14 (19)	15 (46)	0.04‡
RNA Pol III	8 (14)	0 (0)	38 (53)	4 (12)	<0.001‡
Platelet count (× 10^9/liter), median (IQR)	287 (89)	217 (24)	326 (132)	276 (76)	<0.001§
ESR (mm/hour), median (IQR)	18 (31)	30 (4)	19.5 (39)	14 (23)	0.82§
CRP (mg/liter), median (IQR)	0.5 (0.6)	0.25 (0)	0.6 (0.8)	0.5 (0.5)	0.20§

* ACA = anticentromere; ANA = antinuclear antibody; CRP = C-reactive protein; dcSSc = diffuse cutaneous SSc; ESR = erythrocyte sedimentation rate; IQR = interquartile range; IS = intrinsic subset; lcSSc = limited cutaneous SSc; RNA Pol III = RNA polymerase III; Scl-70 = anti-topoisomerase I; SSc = systemic sclerosis.
† Analysis of variance.
‡ Fisher's exact test.
§ Kruskal-Wallis test.

With regard to pulmonary findings, the Fibroproliferative IS had significantly more lung disease compared with other ISs, with 91% of patients having radiographic ILD present on HRCT (vs. Inflammatory 65% and Normal-like 62%; $P = 0.038$) despite having shorter disease duration than the Normal-like IS ($P = 0.03$) and comparable disease duration with the Inflammatory IS ($P = 0.22$). In contrast, there was no significant difference of radiographic ILD among SSc patients classified traditionally, (dcSSc 76% vs. lcSSc 67%; $P = 0.73$) (Figure 1B). Within traditional cutaneous classification, the pulmonary findings of the Fibroproliferative IS persisted. Among dcSSc patients, the dcSSc/Fibroproliferative IS had the highest TLS (12.5 ± 9.7) and higher positive radiographic ILD (89%) compared with other ISs ($P = 0.009$ and $P = 0.04$, respectively), despite only 54% of the group being Scl-70 positive. Among lcSSc patients, all lcSSc/Fibroproliferative patients had positive ILD diagnosis on HRCT compared with lcSSc/Normal-like (62%) and lcSSc/Inflammatory (67%) ($P < 0.001$) despite only 40% of patients being Scl-70 positive.

Utilizing multivariable modeling, IS was significantly associated with ILD presence ($P = 0.016$), with the Fibroproliferative IS having a higher likelihood of positive radiographic ILD compared with Normal-like (odds ratio [OR] = 5.58, 95% CI 1.29, 46.90, $P = 0.01$) than Inflammatory IS versus Normal-like (OR = 1.33, 95% CI 0.45, 3.94, $P = 0.38$). Although traditional cutaneous classification was not significantly associated with increased TLS ($P = 0.31$) or positive radiographic ILD ($P = 0.64$), Scl-70 positivity had a strong association with both ($P < 0.001$ and $P < 0.001$) and was associated with lower DLCO% ($P = 0.01$). Neither IS nor Scl-70 positivity was associated with lower FVC% ($P = 0.43$ and $P = 0.47$, respectively) (Table 5).

Echo findings including left ventricular ejection fraction, left ventricular mass, tricuspid annular plane systolic excursion, right ventricular fractional area change, pulmonary artery systolic pressure, and diastolic dysfunction, were similar among different IS groups ($P > 0.1$) as well as among lcSSc and dcSSc patients ($P > 0.1$). The Normal-like IS demonstrated a trend toward increased prevalence of DD; however, the result was not significant ($P = 0.19$).

Patient-reported outcome instruments. The PROMIS-29 questionnaire assesses 7 health domains—physical

Table 3. Skin, pulmonary, and cardiac findings of SSc patients by IS and traditional cutaneous classification*

	IS classification					Traditional cutaneous classification		
	Normal-like	Limited	Inflammatory	Fibroproliferative	*P*	lcSSc	dcSSc	*P*
Distribution	N = 58	N = 2	N = 72	N = 33		N = 50	N = 115	
mRSS	8.6 ± 5.9	5.0 ± 2.8	22.1 ± 9.9	12.3 ± 7.1	<0.001†	5.7 ± 3.0	19.4 ± 9.4	<0.001‡
PFT	N = 53	N = 2	N = 69	N = 33		N = 45	N = 112	
FVC%	82 ± 18	77 ± 46	79 ± 18	75 ± 16	0.41†	84 ± 18	78 ± 18	0.04‡
FEV1%	82 ± 17	86 ± 16	82 ± 14	76 ± 16	0.42†	84 ± 17	80 ± 15	0.13‡
TLC%	89 ± 19	94 ± 51	88 ± 17	82 ± 18	0.35†	92 ± 19	85 ± 19	0.06‡
DLCO%	69 ± 20	50 ± 35	72 ± 20	63 ± 22	0.15†	71 ± 21	68 ± 21	0.38‡
HRCT	N = 35	N = 2	N = 48	N = 23		N = 31	N = 77	
Fibrosis score	3.6 ± 4.1	6.5 ± 4.9	2.2 ± 2.9	4.3 ± 4.6	0.08†	3.6 ± 3.6	3.0 ± 3.9	0.47‡
GGO score	6.2 ± 5.4	5.5 ± 2.1	4.3 ± 4.4	7.4 ± 5.4	0.09†	5.7 ± 5.0	5.5 ± 5.1	0.87‡
TLS	9.2 ± 8.6	12.0 ± 7.1	6.5 ± 6.4	11.7 ± 9.2	0.06†	8.8 ± 7.8	8.5 ± 8.1	0.86‡
Radiographic ILD present	21 (62)	2 (100)	31 (65)	21 (91)	0.04§	21 (67)	31 (76)	0.73¶
Echo	N = 42	N = 2	N = 48	N = 26		N = 38	N = 80	
LVEF (%)	63 ± 7	60 ± 3	62 ± 5	62 ± 5	0.85†	63 ± 7	62 ± 5	0.47‡
LV mass (g/m^2)	77 ± 18	62 ± 2	81 ± 25	79 ± 18	0.60†	76 ± 16	79 ± 23	0.43‡
TAPSE (cm)	2.2 ± 0.4	2.0 ± 0.1	2.2 ± 0.4	2.1 ± 0.4	0.68†	2.1 ± 0.4	2.2 ± 0.5	0.71‡
RV FAC (%)	43 ± 5	43 ± 7	44 ± 5	43 ± 5	0.99†	42 ± 6	44 ± 5	0.20‡
PASP (mm Hg)	32 ± 9	41 ± 16	31 ± 10	28 ± 8	0.15†	32 ± 11	30 ± 9	0.39‡
DD, n (%)	23 (55)	0 (0.0)	19 (39)	9 (35)	0.20§	17 (45)	34 (43)	0.82¶

* Values are the mean ± SD or the number (%). dcSSc = diffuse cutaneous SSc; DD = diastolic dysfunction; DLCO% = diffusion for carbon monoxide percent predicted; echo = echocardiogram; FEV1 = forced expiratory volume in 1 second; FVC% = forced vital capacity percent predicted; GGO = ground glass opacification; HRCT = high-resolution chest computed tomography; ILD = interstitial lung disease; IS = intrinsic subset; lcSSc = limited cutaneous SSc; LV = left ventricle; LVEF = left ventricular ejection fraction; mRSS = modified Rodnan skin score; PASP = pulmonary artery systolic pressure; PFT = pulmonary function test; RV FAC = right ventricular fractional area change; SSc = systemic sclerosis; TAPSE = tricuspid annular plane systolic excursion; TLC = total lung capacity; TLS = total lung disease score.
† Analysis of variance.
‡ Student's *t*-test.
§ Fisher's exact test.
¶ Pearson's chi-square test.

function, anxiety, depression, fatigue, sleep disturbance, satisfaction with social role, and pain—and includes 4 questions (items) for each domain plus a 1 to 10 pain scale. There were significant differences in scores for 4 of 7 domains for patients classified by IS (physical function, $P = 0.02$; sleep disturbance, $P = 0.04$; satisfaction with social participation, $P = 0.013$; and pain, $P = 0.002$) but differences in only 1 domain for patients traditionally classified (social participation, $P = 0.001$) (Supplementary Table S1, available on the *Arthritis Care & Research* website at https://onlinelibrary.wiley.com/doi/10.1002/acr.24998). Patients in the Inflammatory and Fibroproliferative ISs reported more limitations in physical function, sleep disturbances, and pain and less satisfaction with social participation compared with the Normal-like IS. The FACIT-Dyspnea and SGRQ, which were limited by fewer responses, did not demonstrate significant differences between IS or traditional cutaneous classification (Supplementary Table S1, at https://onlinelibrary.wiley.com/doi/10.1002/acr.24998).

DISCUSSION

SSc is a clinically heterogeneous disease, with some patients demonstrating relatively normal organ function and low symptom burden, whereas others experience life-threatening and disabling disease. Recent clinical trial results for SSc skin disease have been disappointing, with patients who were randomized to several promising agents (e.g., abatacept, lenabasum, tocilizumab) failing to meet the primary clinical end point (3,4,13). One reason for negative trial results may be our inability to identify SSc patients with similar phenotypes for whom targeted treatments are more likely to be effective. The study aim was to elucidate the clinical phenotypes of SSc patients classified according to the newer IS classification system based upon skin gene expression and examine how they compare with and add to traditional cutaneous and antibody status classifications. The goal was to determine whether IS classification may be advantageous for identifying SSc patients with similar disease phenotype. We demonstrate that IS classification identifies distinct clinical SSc phenotypes that have both shared and unique features with clinically available subtypes.

Utilizing genome-wide gene expression analysis of the skin, Milano et al were the first to classify patients among 4 ISs that appeared biologically relevant and distinct from the traditional cutaneous classification system (5). The Inflammatory IS most highly expressed genes associated with the presence of inflammatory infiltrates and increased immune response (5). In the Fibroproliferative IS, genes associated with cell

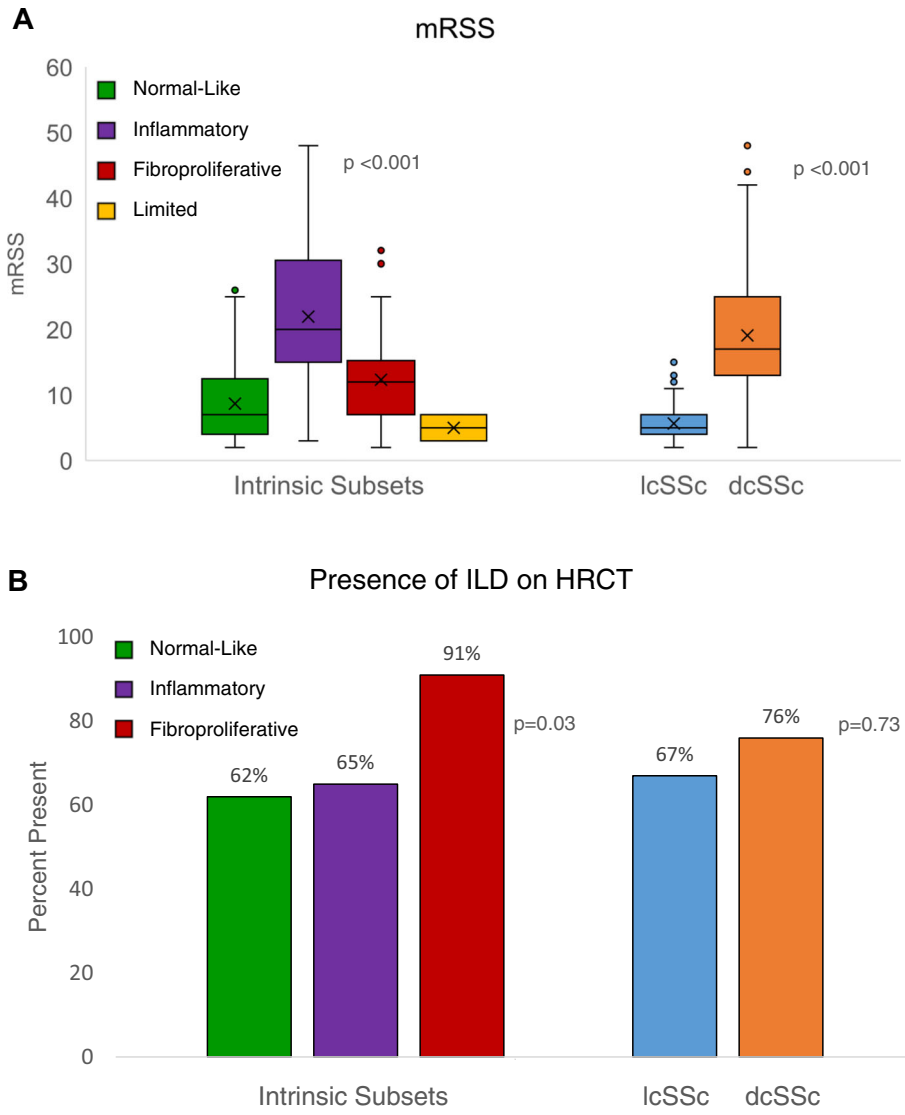

Figure 1. Skin and pulmonary manifestations in systemic sclerosis (SSc) patients by intrinsic subset (IS) compared with traditional cutaneous classification. **A**, Comparison of modified Rodnan skin thickness score (mRSS) between ISs and traditional classification. **B**, Comparison of prevalence of interstitial lung disease (ILD) on high-resolution chest computed tomography (HCRT) between IS and traditional classification. dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc.

proliferation were highly expressed and, interestingly, genes associated with fatty acid and lipid synthesis were downregulated, which is a known SSc hallmark and a putative fibrotic pathogenic mechanism (21,22). Recent studies have sought to understand the clinical implications of ISs and how they may impact treatment decisions (8,9,12). However, many of these studies have been limited by small sample size and lack of comprehensive patient-level data. This study utilized high-quality prospectively collected patient-level information, including echo and HRCT data interpreted according to standardized research protocols.

When compared with Normal-like and Limited IS, the Fibroproliferative and Inflammatory ISs appeared to have more severe SSc as assessed by mRSS, PFTs, HRCT, and acute phase reactants, although each with a distinct phenotype. The Fibroproliferative IS consisted of 73% dcSSc patients, and although not significant, a quarter of the Fibroproliferative patients were Black, which in epidemiologic studies has been associated with more severe SSc and worse prognosis (23,24). Moreover, the Fibroproliferative IS had greater radiographic ILD prevalence and had higher TLS and lower FVC% and DLCO% than the Inflammatory IS, despite there being more dcSSc patients in the latter IS and both ISs having similar disease durations. The Inflammatory IS, which consisted of 90% dcSSc patients, had the highest platelet count and CRP level compared with other ISs and a significantly higher mRSS than the other ISs and the dcSSc group. Taken together, the Fibroproliferative IS appears to represent patients with more lung fibrosis in whom antifibrotic treatments may be

Table 4. Clinical characteristics of SSc patients classified first by traditional cutaneous classification and then by IS*

	lcSSc (N = 50)					dcSSc† (N = 115)			
	Normal-like	Limited	Inflammatory	Fibroproliferative	*P*	Normal-like	Inflammatory	Fibroproliferative	*P*
Distribution	32 (64.0)	2 (4.0)	7 (14.0)	9 (18.0)	<0.001‡	26 (22.6)	65 (56.5)	24 (20.9)	<0.001‡
Skin score	N = 32	N = 2	N = 7	N = 9		N = 26	N = 65	N = 24	
mRSS	5.8 ± 3.2	5.0 ± 2.0	6.1 ± 2.8	5.1 ± 2.2	0.77§	12.2 ± 6.3	23.8 ± 8.8	15 ± 6.2	<0.001§
Antibodies	N = 32	N = 2	N = 7	N = 9		N = 26	N = 65	N = 24	
ACA	10 (20.0)	0 (0.0)	1 (2.0)	2 (4.0)	0.02‡	1 (0.8)	2 (1.7)	0 (0.0)	0.79‡
Scl-70	7 (14.0)	0 (0.0)	1 (2.0)	2 (4.0)	0.25‡	8 (6.9)	13 (11.3)	13 (11.3)	0.007‡
RNA Pol III	1 (2.0)	0 (0.0)	1 (2.0)	0 (0.0)	0.79‡	7 (6.1)	37 (32.1)	4 (3.5)	<0.001‡
PFT	N = 28	N = 2	N = 6	N = 9		N = 24	N = 64	N = 24	
FVC%	83 ± 18	50 ± 25	85 ± 14	83 ± 10	0.96§	79 ± 16	79 ± 18	70 ± 15	0.11§
DLCO%	71 ± 19	41 ± 11	82 ± 20	66 ± 19	0.13§	66 ± 19	70 ± 19	61 ± 22	0.13§
HRCT	N = 17	N = 2	N = 6	N = 5		N = 18	N = 42	N = 18	
Fibrosis score	3.5 ± 3.9	6.5 ± 3.5	1.3 ± 1.5	3.6 ± 2.6	0.30§	3 ± 3.9	2.35 ± 3.0	4.5 ± 4.9	0.05§
GGO score	5.8 ± 5.9	5.5 ± 1.5	3.2 ± 4.5	5.0 ± 2.4	0.49§	5.4 ± 4.5	4.45 ± 4.6	8.1 ± 5.5	0.007§
TLS	8.5 ± 8.9	12.0 ± 5.0	4.5 ± 3.9	8.6 ± 4.45	0.45§	8.4 ± 7.9	6.81 ± 6.6	12.5 ± 9.7	0.009§
Radiographic ILD present	10 (62)	2 (100)	4 (67)	5 (100)	<0.001‡	11(68)	27 (64)	16 (89)	0.04‡
Echo	N = 24	N = 2	N = 6	N = 6		N = 18	N = 42	N = 20	
TAPSE (cm)	2.1 ± 0.4	2.01 ± 0.1	2.2 ± 0.4	2.1 ± 0.1	0.89§	2.2 ± 0.5	2.19 ± 0.4	2.0 ± 0.4	0.14§
PASP (mm Hg)	34 ± 11	41 ± 11	30 ± 10	27 ± 3	0.19§	31 ± 4	31 ± 9	29 ± 9	0.48§
Laboratories	N = 30	N = 2	N = 6	N = 9		N = 25	N = 62	N = 23	
Platelet count (× 10^9/liter)	275 ± 60	217 ± 12	278 ± 85	278 ± 45	0.97¶	283 ± 49	346 ± 104	269 ± 55	<0.001¶
ESR (mm/hour)	24 ± 20	30 ± 2	16 ± 18	30 ± 34	0.47¶	22 ± 14	27 ± 22	22 ± 23	0.33¶
CRP (mg/liter)	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.6 ± 0.26	0.06¶	1.7 ± 3.1	4.6 ± 13.9	0.9 ± 0.7	0.11¶

* Values are the mean ± SD or the number (%). ACA = anticentromere; CRP = C-reactive protein; dcSSc = diffuse cutaneous SSc; DLCO% = diffusion capacity of carbon monoxide percent predicted; echo = echocardiogram; ESR = erythrocyte sedimentation rate; FVCT = forced vital capacity percent predicted; GGO = ground glass opacification; HRCT = high-resolution chest computed tomography; ILD = interstitial lung disease; IS = intrinsic subset; lcSSc = limited cutaneous SSc; mRSS = modified Rodnan skin score; PASP = pulmonary artery systolic pressure; PFT = pulmonary function test; RNA Pol III = RNA polymerase III; Scl-70 = anti-topoisomerase I; SSc = systemic sclerosis; TAPSE = tricuspid annular plane systolic excursion; TLS = total lung disease score.
† Within the dcSSc subgroup, no patients were classified as Limited IS.
‡ Fisher's exact test.
§ Analysis of variance.
¶ Kruskal-Wallis test.

Table 5. Clinical predictors of SSc skin and pulmonary outcomes*

Predictor variables	Skin and pulmonary outcome variables				
	mRSS, β (95% CI)†, N = 163	TLS, β (95% CI)†, N = 106	FVC%, β (95% CI)†, N = 155	DLCO%, β (95% CI)†, N = 155	Radiographic ILD, OR (95% CI)‡, N = 105
IS					
Inflammatory	8.15 (5.24, 11.06)	−1.47 (−5.03, 2.09)	−0.67 (−8.05, 6.72)	2.89 (−5.81, 11.6)	1.33 (0.45, 3.94)
Proliferative	1.30 (−1.89, 4.49)	0.16 (−4.01, 4.33)	−4.93 (−12.89, 3.03)	−3.96 (−13.38, 5.47)	5.58 (1.29, 46.90)
Normal-like (ref.)	–	–	–	–	–
SSc subtype: dcSSc vs. lcSSc	9.42 (6.54, 12.30)	1.85 (−1.69, 5.38)	−5.61 (−13.18, 1.95)	−6.36 (−15.3, 2.57)	1.32 (0.46, 3.68)
SSc disease duration: >36 mo vs. ≤36 mo	0.48 (−1.95, 2.91)	4.34 (1.14, 7.55)	−1.93 (−8.01, 4.16)	−6.09 (−13.32, 1.14)	1.37 (0.50, 3.66)
Scl-70: positive vs. negative	2.19 (−0.33, 4.71)	7.47 (4.12, 10.82)	−2.12 (−9.3, 5.06)	−8.78 (−17.25, −0.32)	13.19 (2.4, 403.4)
RNA Pol III: positive vs. negative	2.62 (−0.09, 5.34)	1.39 (−1.90, 4.68)	−1.29 (−8.75, 6.16)	1.19 (−7.61, 9.99)	1.57 (0.49, 5.08)
ACA: positive vs. negative	–	–	5.9 (−4.49, 16.29)	2.75 (−9.39, 14.89)	–
Immunosuppressant (current): yes vs. no	0.08 (−3.13, 3.3)	2.04 (−1.66, 5.74)	−2.8 (−10.94, 5.34)	−4.77 (−14.76, 5.22)	–
Smoker (ever): yes vs. no	1.16 (−1.9, 4.22)	0.15 (−3.39, 3.69)	9.05 (1.47, 16.62)	0.85 (−8.29, 9.99)	–

* Shown are the clinical predictors and their associated difference in mRSS, TLS, FVC%, DLCO%, and presence of radiographic ILD as resulting from multiple regression modeling. Cells with no values denote variables that were not included to permit model convergence. ACA = anticentromere; 95% CI = 95% confidence interval; dcSSc = diffuse cutaneous SSc; DLCO % = diffusion capacity for carbon monoxide percent predicted; FVC% = forced vital capacity percent predicted; ILD = interstitial lung disease; IS = intrinsic subset; lcSSc = limited cutaneous SSc; mRSS = modified Rodnan skin score; OR = odds ratio; ref. = reference variable; RNA Pol III = RNA polymerase III; Scl-70 = anti-topoisomerase I; SSc = systemic sclerosis; TLS = total lung disease score.
† Multiple linear regression.
‡ Exact logistic regression.

most effective, whereas the Inflammatory IS identifies patients with more active skin disease in whom drugs targeting innate or adaptive immune responses may be more appropriate.

Patients with dcSSc and Scl-70 positivity are thought to be at higher risk for ILD (2,25). We found the prevalence of radiographic ILD was similar between lcSSc and dcSSc patients. In multivariable models, patients with Scl-70 antibodies compared with those with ACA or RNA Pol III were more likely to have radiographic ILD. Moreover, positive Scl-70 was a better predictor of radiographic ILD compared with IS classification. However, we demonstrate that IS classification identifies additional lcSSc patients (lcSSc/Fibroproliferative) with radiographic ILD independent of antibody status. In fact, 5 lcSSc/Fibroproliferative patients had radiographic ILD within 29.9 ± 20.1 months of disease duration, and only 2 had Scl-70 antibodies. Furthermore, of these lcSSc patients, 3 had normal FVC% (≥80% predicted), and 1 had normal DLCO% (≥60% predicted), and chest HRCT might not have been pursued in these patients. The limitations of PFT has been demonstrated in our previous work involving 265 (188 [71%] with radiographic ILD) SSc patients, in which we showed that 59 (31%) had "normal" FVC% and 65 out of 151 (43%) had "normal" DLCO% (18). Moreover, lcSSc/Fibroproliferative patients compared with lcSSc/Inflammatory and lcSSc/Normal-like patients were more likely to have radiographic ILD ($P < 0.001$). Thus, although Scl-70 antibody appears to be the strongest risk factor for ILD in our cohort and has the benefit of being readily available, ISs may allow identification of an additional at-risk group (i.e., lcSSc/Fibroproliferative) for ILD. Future studies will determine which SSc subtype/IS combinations have the highest risk of developing progressive ILD.

Among dcSSc patients, the Fibroproliferative IS also had more severe ILD compared with other ISs. Using granular chest HRCT data, specifically GGO and fibrosis scores (16), we found that dcSSc/Fibroproliferative patients had the highest GGO (8.1 ± 5.5) and fibrosis scores (4.5 ± 4.9), whereas scores were lowest in lcSSc/Inflammatory patients (GGO 3.2 ± 4.5, fibrosis 1.3 ± 1.5). When faced with choosing the most appropriate Food and Drug Administration–approved treatment for SSc-ILD (tocilizumab, an IL-6 receptor antagonist [3], vs. nintedanib, a tyrosine kinase receptor antagonist [26]), tocilizumab might be best for the dcSSc/Fibroproliferative subset patients with high GGO scores, whereas nintedanib may be more effective for patients with high fibrosis scores. The verity of this hypothesis warrants testing.

With regard to skin disease, IS and cutaneous subtype had the strongest association with high mRSS ($P < 0.001$); however, disease duration, Scl-70 positivity, and RNA Pol III positivity were not significantly associated. Among IS and cutaneous classification, dcSSc/Inflammatory patients had the highest mRSS (23.8 ± 8.8) compared with dcSSc/Fibroproliferative (15.0 ± 6.2) and dcSSc/Normal-like (12.2 ± 6.3) ($P < 0.001$). In addition, dcSSc/Inflammatory patients had a higher mean mRSS than either dcSSc (19.4 ± 9.4) or Inflammatory IS (22.1 ± 9.9) alone, suggesting that together, IS and cutaneous classification may identify patients with the most severe skin disease, but determining the predictive ability of the dual classification will be important. Similar to dcSSc/Inflammatory patients, lcSSc/Inflammatory patients had the highest mean mRSS (6.1 ± 2.8) compared with lcSSc/Normal-like (5.8 ± 3.2) and lcSSc/Fibroproliferative (5.1 ± 2.2) groups, although the differences were not statistically or likely clinically significant. However, we propose that consideration be given to treating skin disease in SSc patients who demonstrate an inflammatory skin gene expression signature with anti-inflammatory agents, such as mycophenolate mofetil and abatacept, that have been previously demonstrated to impact mRSS regardless of whether they have lcSSc or dcSSc (10,27). Of course, additional longitudinal analyses are necessary to evaluate the effectiveness of this approach.

Our study findings may help explain the recent negative results of the Phase III Lenabasum trial, in which dcSSc patients were randomized to receive oral lenabasum, a cannabinoid type 2 receptor agonist, versus placebo (4). The primary outcome (the American College of Rheumatology Combined Response Index in diffuse cutaneous systemic sclerosis score [28]), and secondary outcomes, including mRSS, were not met. However, results of post hoc analyses from the previous phase II trial found that patients lacked elevated IL-6 levels, and the majority of patients belonged to the Fibroproliferative IS (29). Because CB2 receptors are primarily located on immune cells, the potential inclusion of Fibroproliferative IS rather than Inflammatory IS patients in the Phase III trial may explain the negative results. Forthcoming analyses of the skin gene expression data will shed light on this.

Study strengths include our multicenter study design and the collection of skin gene expression and comprehensive clinical data for a robust SSc cohort that included patients from 4 large academic centers. We were able to examine several clinical data domains, including serologic, pulmonary, cardiac, and PRO findings, that had not previously been examined. Although we aimed to obtain comprehensive data from all patients, there were a subset of patients lacking either echo (n = 47) or HRCT (n = 57), which is a study limitation. In the present study, we purposefully recruited patients with active skin disease in the opinion of the treating physician. It is possible that a subset of patients classified as lcSSc, especially those with positive Scl-70 antibodies, had progressive skin disease over time and will be subsequently reclassified as having dcSSc. Analyses of longitudinal data are underway that will address this possibility. We utilized a validated IS classifier with an accuracy rate of 87.1%, which introduces the potential for misclassification. However, in comparison with previously utilized IS classifiers, this classifier can be applied to single compared with paired samples to increase utility and feasibility in studies and clinical trials. Overall, we did not correct for

performing multiple statistical tests given the exploratory nature of the analyses and the intention to generate additional testable hypotheses. Moreover, the cross-sectional study design precluded determination of the prognostic value of IS classification. We recognize that our study is not the definitive study on the importance of IS classification, and work is underway analyzing prospectively collected longitudinal data.

In conclusion, we utilized molecular gene expression signatures of the skin to classify SSc patients into distinct ISs that demonstrated unique clinical characteristics and varying disease severity that may provide added value to traditional cutaneous and antibody classifications. In addition to these existing classification models, the IS classification identifies patients with more skin fibrosis (Inflammatory IS) and radiographic ILD (Fibroproliferative IS) with Normal-like and possibly Limited IS patients exhibiting milder disease phenotype. Taken together, these findings demonstrate that the IS classification provides additive value that may be utilized in combination with the traditional cutaneous and serum autoantibody classification to aid clinicians and researchers in clinically impactful SSc patient stratification.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Yang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Yang, Hinchcliff.

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Analysis and interpretation of data. Yang, Lee, Espinoza, Yuan, Agrawal, Whitfield, Hinchcliff.

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