The Effect of Anti-Scl-70 Antibody Determination Method on Its Predictive Significance for Interstitial Lung Disease Progression in Systemic Sclerosis

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Objective. The objective of this study was to assess the predictive significance of anti-Scl-70 (anti-topoisomerase I) antibodies, as determined by three different methods, for decline in forced vital capacity (FVC) within the first year of follow-up in patients with systemic sclerosis (SSc)-related interstitial lung disease (ILD).

Methods. Patients in the Genetics Versus Environment in Scleroderma Outcome Study cohort who had ILD (verified by imaging) and available FVC% at enrollment, plus 12 to 18 months thereafter, were examined. All patients had a disease duration of 5 years or less at enrollment. The annualized percentage change in FVC% at 1 year follow-up was the outcome variable. Anti-Scl-70 antibodies were determined by passive immunodiffusion (ID) against calf thymus extract, chemiluminescent immunoassay (CIA), and line blot immunoassay (LIA).

Results. Ninety-one patients with a mean disease duration of 2.36 years were included. Anti-Scl-70 antibodies by ID predicted a faster rate of FVC% decline ($b = -0.06$, $P = 0.04$). None of the other clinical or serological variables significantly predicted ILD progression. Interestingly, anti-Scl-70 antibodies as determined by CIA and LIA were not significant predictors of FVC decline ($P = 0.26$ and 0.64, respectively). The observed level of agreement between ID and LIA was moderate ($κ = 0.568$), whereas it was good between ID and CIA ($κ = 0.66$).

Conclusion. Anti-Scl-70 antibodies determined by ID predicted faster FVC decline in patients with SSc-related ILD. Notably, both CIA and LIA for the same antibody did not predict rate of FVC decline at their current cutoffs of positivity. The discrepancy observed between anti-Scl-70 antibody assays can have relevant implications for clinical care and trial enrichment strategies in SSc-ILD.

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis of skin and internal organs as well as vasculopathy and immune dysregulation with production of autoantibodies.

Interstitial lung disease (ILD) is currently the primary cause of disease-related mortality from SSc (1). Anti-Scl-70 antibodies (also known as anti-topoisomerase I antibodies) are associated with severity and development of SSc-ILD (2–6). Key clinical trials studying the treatment of this disease manifestation reveal personal fees from Medtelligence, personal fees from Actelion Pharma, grant support and personal fees from Mitsubishi-Tanabe, grant support and personal fees from Boehringer Ingelheim, grant support and personal fees from EICOS, grant support from Corbus, and grant support from Horizon outside the submitted work. Dr. Assassi reports grants from National Institute of Health and grants from the Department of Defense during the conduct of the study; he also reports grants from Momenta, grants and personal fees from Boehringer Ingelheim, grants from Janssen, grants from Scleroderma Research Foundation, personal fees from Corbus, personal fees from Novartis, personal fees from CSL Behring, personal fees from AbbVie, personal fees from AstraZeneca, and personal fees from Integrity CE outside the submitted work. The rest of the authors have nothing to disclose.

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marked progression variability among patients, which blunts the observed treatment effects. Clinical trials examining SSC-ILD typically have a duration of 1 to 2 years (7–10). Hence, there is a growing need to differentiate the patients who are unlikely to progress (nonprogressors) from those with progressive disease.

In the aforementioned studies linking anti-Scl-70 antibodies to ILD severity, these antibodies were determined by immunodiffusion (ID). Recently, newer techniques to identify anti-Scl-70 antibodies have been developed and are used widely in clinical practice and trials, although the prognostic properties of anti-Scl-70 antibodies as determined by these technologies have not been well studied.

The objective of this study was to identify clinical and serological factors (especially anti-Scl-70 antibodies determined by different methods) that predict faster forced vital capacity (FVC) decline within the first year of follow-up in SSC-ILD to inform individualized care in routine clinical practice and to aid enrichment strategies in clinical trials.

PATIENTS AND METHODS

Study population. The Genetics Versus Environment in Scleroderma Outcome Study (GENISOS) is an ethnically diverse prospective multicenter study (11,12) with the following inclusion criteria: 1) age 18 years or older, 2) SSC diagnosis according to the American College of Rheumatology 1980 classification criteria (13), and 3) disease onset (defined as the first non-Raynaud symptom) within the previous 5 years.

All patients enrolled in the GENISOS cohort (11) at the time of analysis who had the following characteristics were included: 1) ILD verified by imaging and 2) pulmonary function tests (PFTs) at enrollment and a second set at 12 to 18 months. Although not used as an inclusion criterion, all patients also fulfilled the 2013 American College of Rheumatology/European League Against Rheumatism classification criteria for SSC (14). Immunosuppressive therapy was examined both at baseline and at the 1-year follow-up visit (defined as treatment with any immunosuppressive agents except for hydroxychloroquine or prednisone at ≤5 mg daily).

Autoantibodies. Presence of antinuclear antibodies was investigated in all patients by using indirect immunofluorescence on Hep-2 cells as the antigen substrate in the rheumatology laboratory of the University of Texas Health Science Center at Houston. Anticentromere antibodies (ACAs) were determined by the fluorescence staining on Hep-2 cells. Anti-Scl-70, anti-U1-RNP, anti-SSA (anti-Ro), and anti-SSB (anti-La) antibodies were determined by passive ID against calf thymus extract with commercial kits (Inova Diagnostics). Anti-RNA polymerase III antibodies were determined by enzyme-linked immunosorbent assay (ELISA) (Medical & Biological Laboratories, Co. Ltd). Furthermore, anti-Ro52 antibodies were determined by line blot immunoassay (LIA) (EUROLINE; Euroimmun AG). Additionally, anti-Scl-70 antibodies were also determined by chemiluminescence immunoassay (CIA) (BIO-FLASH; Inova Diagnostics) (15) and LIA (EUROLINE; Euroimmun AG) at the Cumming School of Medicine in Calgary. CIA is interpreted with the help of a fully automated chemiluminescent analyzer (BIO-FLASH; Inova Diagnostics) on the basis of chemiluminescence units (CUs). CUs are directly related to the titer of the autoantibody in the patient sample. Increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in CUs, which are proportional to the amount of antibodies found in the sample. The analytical measuring range of the assay is 3.8 CUs to 969.8 CUs (16). Less than 20 CUs is interpreted as a negative result and greater than or equal to 20 CUs is interpreted as a positive result (17).

The EUROLINE test kit provides a qualitative in vitro assay for human autoantibodies of the immunoglobulin G (IgG) class to different antigens in serum or plasma samples. The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, diluted patient samples are incubated with the immunoassay (IB) strips. In the case of positive samples, the specific autoantibodies will bind to the corresponding antigen. To detect the bound antibodies, a second incubation is performed by using an enzyme-labeled anti-human IgG (enzyme conjugate) to catalyze a color reaction. The test is interpreted by a software (EUROLineScan) on the basis of signal intensity measured as grey scale units. The Euroimmun EUROLINE SSC (Nucleoli) Profile IgG autoantibody line assay kit was used to determine the presence of anti-Scl-70 antibodies by LIA (18). A level of 11 or more is considered a positive result, and less than 11 is interpreted as a negative result. A higher signal intensity indicates a higher amount of antibodies in the evaluated sample.

| Characteristic | All (N = 91) |
|---------------|-------------|
| Age at disease onset (years) | 53.4 ± 10.92 |
| Disease duration (years) | 2.36 ± 1.49 |
| Sex (male) | 23 (25%) |
| African American | 20 (21%) |
| Disease type (limited) | 41 (46%) |
| Immunosuppression at baseline | 46 (51%) |
| Immunosuppression at follow-up | 51 (56%) |
| FVC% at baseline | 74 ± 17.25 |
| mRSS | 16.58 ± 11.31 |
| Anti-Scl-70 ID | 21 (23%) |
| Anti-Scl-70 LIA | 27 (31%) |
| Anti-Scl-70 CIA | 23 (26%) |
| RNA polymerase III | 24 (26%) |
| Anti-RNP | 4 (4%) |
| ACA | 4 (4%) |
| Anti-Ro52 | 33 (36%) |

Abbreviations: ACA, anticentromere antibody; anti-Scl-70, antitopoisoerase I antibody; CIA, chemiluminescent immunoassay; FVC, forced vital capacity; ID, immunodiffusion; LIA, line blot immunoassay; mRSS, modified Rodnan skin score.

a Results for this antibody were missing in three patients, of whom one patient had anti-Scl-70 by ID.
Clinical manifestations. Demographic and clinical data were entered by using a standard abstract form. Age, sex, disease type, disease duration (calculated from the onset of both Raynaud and the first non-Raynaud phenomenon symptoms attributable to SSc), and the modified Rodnan skin score (mRSS) at the time of study entry were recorded. Disease type (limited cutaneous vs diffuse cutaneous SSc) was defined on the basis of the extent of skin involvement as assessed by the physician at enrollment (19).

For this particular study, only patients with imaging changes consistent with scleroderma-related fibrosis or ILD were included. The abnormalities that were observed in these patients included increased basilar interstitial markings on the chest x-ray or honeycombing, increased interstitial markings, and/or ground glass opacity on chest computed tomography (CT) or high-resolution CT (HRCT) of the chest. Twenty-one patients fulfilled this criterion on the basis chest x-ray abnormalities alone; 71 patients had an abnormal HRCT result, of whom 29 also had an abnormal chest x-ray result. PFTs were obtained on enrollment and at the 12- to 18-month follow-up. All PFT data were reviewed by a pulmonologist (RME-Y-M), and data that did not fulfill the American Thoracic Society/European Respiratory Society criteria were excluded.

Predicted FVC values were calculated according to the patient’s age, height, weight, sex, and ethnicity by using consistent reference values (20).

Data analysis. FVC, expressed as a percentage of predicted value, was used as a surrogate of ILD progression. Annualized percentage change in FVC% at the 1-year follow-up was calculated for all patients by using the following formula: \( \frac{(FVC\%_{PFT1} - FVC\%_{PFT0})/(time\ PFT1 - time\ PFT0)}{PFT0} \), in which PFT0 = PFT at the enrollment visit, and PFT1 = PFT at the 1-year follow-up visit. This formula accounts for differences in the baseline FVC value. Baseline demographic and clinical variables (including SSc-related autoantibodies, as detailed above) were investigated as potential predictors of FVC change. The annualized percentage FVC change was considered as the

![Figure 1. Distribution of annualized percentage change in forced vital capacity % (FVC%).](image)

| Variables                  | b (mean difference) | 95% CI          | P     |
|----------------------------|---------------------|-----------------|-------|
| African American           | -0.009              | -0.07 to 0.05   | 0.75  |
| Disease type (limited)     | -0.04               | -0.09 to 0.008  | 0.09  |
| Disease duration           | -0.0007             | -0.01 to 0.01   | 0.93  |
| Immunosuppression at baseline | -0.03         | -0.08 to 0.19   | 0.21  |
| Immunosuppression at follow-up | -0.002       | -0.05 to 0.05   | 0.93  |
| mRSS                       | 0.001               | -0.0005 to 0.004 | 0.13  |
| SOB VAS                    | 0.002               | -0.005 to 0.01  | 0.49  |
| ACA                        | -0.03               | -0.14 to 0.07   | 0.55  |
| Anti-RNA polymerase III    | 0.002               | -0.05 to 0.06   | 0.92  |
| Anti-RNP                   | 0.01                | -0.11 to 0.13   | 0.84  |
| Anti-Ro52                  | 0.01                | -0.04 to 0.07   | 0.61  |
| Anti-Th/To                 | 0.01                | -0.16 to 0.19   | 0.84  |
| Anti-Scl-70 CIA            | -0.03               | -0.09 to 0.02   | 0.26  |
| Anti-Scl-70 LIA            | -0.01               | -0.08 to 0.05   | 0.64  |
| Anti-Scl-70 ID             | -0.06               | -0.12 to 0.04   | 0.04  |

Abbreviations: ACA, anticientromere antibody; anti-Scl-70, antitopoiso¬merase I antibody; CI, confidence interval; CIA, chemiluminescent immunoassay; FVC, forced vital capacity; ID, immunodiffusion; LIA, line blot immunoassay; mRSS, modified Rodnan skin score; SOB VAS, shortness of breath visual analog scale.
outcome or dependent variable, and the clinical and demographic variables were incorporated into univariable and multivariable linear models as independent variables. Only variables that reached a \( P \) value less than 0.2 after the univariable analysis were included in the multivariable model. Backward stepwise selection was used to construct multivariable linear regression models. Variables with a \( P \) value less than 0.05 in multivariable models were retained for the final model. We also calculated concordance score and \( \kappa \) statistics to compare the agreement between different techniques of anti-Scl-70 antibody measurements. Analysis was performed by using the Stata 12 (Stata Corp LP) statistical package.

RESULTS

A total of 91 patients were included in this study, of whom 23 were male, 21 were African American, and 51 had diffuse disease. Twenty-one were positive for anti-Scl-70 antibodies per ID; 27, per LIA; and 23, per CIA. The mean disease duration, based on the first non-Raynaud disease manifestation at enrollment, was 2.36 years. The baseline characteristics of the patients included in this study are listed in Table 1.

The four patients who were positive for ACAs were negative for anti-Scl-70 antibodies by all three methods. None of the patients had anti-fibrillarin antibodies.

On follow-up, 50 patients (54.9%) had experienced a numeric worsening in their FVC. Figure 1 shows the distribution of the annualized percent change in FVC% in this cohort. As shown in Table 2, anti-Scl-70 antibodies by ID was the only clinical variable that significantly predicted a faster rate of FVC decline at the 1-year follow-up (\( b = 0.06, P = 0.04 \)). We also attempted to build a multivariable model using the aforementioned variable selection strategy (see data analysis section). However, none of the additional variables beyond anti-Scl-70 antibodies reached a \( P \) value less than 0.05 in the multivariable model.

Interestingly, anti-Scl-70 antibody determinations performed by CIA and LIA were not significant predictors of FVC decline (\( P = 0.26 \) and 0.64, respectively). Moreover, all other autoantibodies and clinical variables were not predictive of FVC decline (Table 2).

**Table 3.** Number and percentage of patients with an annualized percentage decline in FVC% \( \geq 5\% \) according to anti-Scl-70 status

| Anti-Scl-70 status | Total number | Number of patients with annualized percentage decline in FVC% \( \geq 5\% \) | Percentage of patients with annualized percentage decline in FVC% \( \geq 5\% \) |
|--------------------|--------------|-------------------------------------------------|----------------------------------|
| Positive by ID     | 21           | 9                                               | 43%                              |
| Negative by ID     | 70           | 19                                              | 27%                              |
| Positive by CIA but negative by ID | 7          | 2                                               | 29%                              |
| Positive by LIA but negative by ID | 11         | 3                                               | 27%                              |

Abbreviations: anti-Scl-70, anti-topoisomerase I antibody; CIA, chemiluminescent immunoassay; FVC, forced vital capacity; ID, immunodiffusion; LIA, line blot immunoassay.
Among patients with an annualized percentage decline in FVC% of 5% or more, 32% were positive for anti-Scl-70 antibodies by ID; 30%, by LIA; and 26%, by CIA. Among those who had an FVC% decline of less than 5%, a total of 19% were positive for anti-Scl-70 antibodies by ID; 31%, by LIA; and 26%, by CIA (see Figure 2). As shown in Table 3, 43% of patients with anti-Scl-70 antibody positivity by ID had an annualized percentage decline in FVC% of greater than or equal to 5%, whereas the percentage of patients with this amount of FVC decline in those who were positive for anti-Scl-70 antibodies by CIA or LIA, but negative by ID, was lower (29% and 27%, respectively) and was in a similar range as the percentage of patients negative for anti-Scl-70 antibodies by ID (27%).

Percentage agreement for anti-Scl-70 antibodies determined by ID and CIA was 87.5%; by ID and LIA, 83%; and by CIA and LIA, 86.4%. The observed level of agreement between ID and LIA was moderate ($\kappa = 0.568$), whereas it was good between ID and CIA ($\kappa = 0.66$) (21). Of the 21 patients who were positive for anti-Scl-70 antibodies by ID, four were negative by LIA and four were negative by CIA (see Table 4).

### DISCUSSION

Anti-Scl-70 antibodies determined by ID was the only clinical variable that predicted faster FVC% decline in patients with SSc-related ILD. Notably, both CIA and IB assay for the same antibody were not predictive of a higher rate of FVC% decline at their current cutoffs of positivity.

Autoantibody formation is one of the hallmarks of SSc. Several studies have shown that the autoantibodies found in patients with SSc carry considerable value in diagnosis and in predicting various clinical outcomes. Our current study supports the role of anti-Scl-70 antibodies by ID as a prognostic biomarker for SSc-ILD. Anti-Scl-70 antibody was first described in 1979 and observed to target a then unknown chromatin-associated protein initially designated as Scl-70 and later identified as DNA topoisomerase I (hence the two names used at the present time) (3,22). This enzyme is essential for DNA replication, transcription, and recombination by introducing transient breaks into the helix, which causes relaxation of the torsional tension of DNA, allowing other enzymes to reach it (23,24). Anti-Scl-70 antibodies are found in approximately 20% of patients with SSc; however, owing to ethnic differences, the prevalence can range from 20% to 46% depending on the population being studied (3, 6, 8, 25). Studies such as the Scleroderma Lung Study II, which recruited patients with SSc with clinically significant ILD, reported a higher rate anti-Scl-70 antibody positivity (46% as determined by ID) (8).

Anti-Scl-70 antibodies as determined by ID are usually not found in healthy individuals, in family members of patients with SSc, or in individuals with other connective tissue disorders. Therefore, they are highly specific for SSc. In a large serological study of first-degree relatives ($n = 1005$) of patients with SSc, spousal controls ($n = 186$), and unrelated controls ($n = 644$), anti-Scl-70 antibodies as determined by ID were present in 20.4% of patients with SSc, but none of the first-degree relatives or control participants (including spousal controls) had these antibodies (6,25). These findings underscore the specificity of anti-Scl-70 antibodies as determined by ID.

There is a well-described association of anti-Scl-70 antibodies with severity of skin involvement, diffuse disease type, and ILD (5). Furthermore, this antibody predicted increased mortality risk in an international multicenter study as well as in the GENILOS cohort (4,11,26). The above-mentioned studies all used ID to determine the presence of anti-Scl-70 antibodies. However, for reasons discussed below, at the present time, other assay types are commonly used for the detection of anti-Scl-70 antibodies both in clinical practice and trials.

The classic technique for anti-Scl-70 antibody determination is ID against calf or rabbit thymus extract. ID consists of a diffusion of an antigen and antibody in two dimensions from separate sources into a gel, generally agarose (24). This method is the gold standard but has some disadvantages, including that the methodology is labor intensive and time consuming (usually requires 2-3 days for completion); thus it cannot be automated and performed quickly in a large number of samples. Furthermore, the results are mainly qualitative rather than quantitative (3,24).

To solve the perceived deficiencies of ID, other assays, including IB, ELISA, and CIA have been introduced. ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, and antibodies. Based on the principle of antibody–antibody interaction, the CIA is a variation of the standard ELISA in which the final reaction emits photons of light instead of developing a visible color for interpretation. The luminescent signal produced is then captured by an instrument that then translates it into numeric data that can be interpreted. These instruments can handle multiple samples and yield fast results (15).

IB, more commonly known as Western blot, is a method designed to detect proteins in a given sample of tissue or cell extract. It uses gel electrophoresis to separate denatured proteins according to their molecular weight. The proteins are then transferred onto a membrane, where they are probed by using specific antibodies or other antibody preparations. LIA is a variation of the classic IB that enables simultaneous testing of multiple antibodies. The antigens are placed on nitrocellulose as narrow lines;

### Table 4. Test results per assay type and their concordance

| Test type and result | LIA positive N/A N/A N/A 4 57 | LIA negative N/A N/A N/A 4 57 | CIA positive N/A N/A N/A 4 57 | CIA negative N/A N/A N/A 4 57 |
|----------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| ID positive          | 16                              | 4                               | 16                              | 4                               |
| ID negative          | 11                              | 57                              | 7                               | 61                              |
| LIA positive         | N/A                             | N/A                             | 19                              | 8                               |
| LIA negative         | N/A                             | N/A                             | 4                               | 57                              |

Abbreviations: CIA: chemiluminescent immunoassay; ID: immunodiffusion; LIA: line blot immunoassay; N/A, not applicable.
therefore, different antigens can be screened and typed on the same sample (24).

All of these assays (ELISA, CIA, IB, and LIA) have strengths and weaknesses, and the reliability of the results obtained depends on the characteristics of the technique and the antigen source. Notably, most of these commercially available assays have replaced the originally used antigen, native topoisomerase I protein, with fusion proteins, which can introduce further variability (27).

Few previous studies have compared the accuracy of different immunoassay modalities for anti-Scl-70 antibodies. One group investigated serum samples from 409 patients with SSc and controls using classic ID, IB, and ELISA methods with purified topoisomerase I as the antigen for all of them. They found that ELISA was more sensitive than ID and was more specific than IB but noted that IB might identify additional autoantibodies (23).

Another study compared multiple commercially available ELISA and IB assays for anti-Scl-70 antibodies in SSc and found comparable sensitivity and specificity between the various assays (27). We did not perform classic ELISA but as previously discussed, CIA is a variation of ELISA.

An Italian group previously studied the accuracy of CIA by Menarini Diagnostics (Florence, Italy) and compared it with ID and found a 94% $\kappa$ agreement (28). In a study of 145 Belgian patients with SSc and 277 disease controls (individuals with other rheumatic diseases), there was very good agreement between LIA by EUROLINE and ID for anti-Scl-70 antibodies ($\kappa = 0.91$) (21). The clinical correlates and prognostic significance of anti-Scl-70 antibodies determined by EUROLINE was not investigated in this study (29).

Another group previously studied both LIA (EUROLINE) and ELISA in patients with SSc and reported a significant correlation between LIA signal strengths and antibody levels as detected by ELISA ($P < 0.0005$). $\kappa$ Was not calculated. They also reported that in this sample, anti-Scl-70 antibody positivity was associated with ILD. However, this study did not compare LIA with ID, nor did it investigate the predictive significance of anti-Scl-70 antibodies by LIA for FVC decline over time (30). Finally, a recent study compared performance of anti-Scl-70 antibody testing by multiple-bead assay with that by ELISA, followed by ID for those samples positive by ELISA. Of 129 participants who were positive for anti-Scl-70 antibodies by multiple-bead assay, 51 were also positive by ELISA, and only 21 were positive by ELISA and ID. More importantly, 26.4% of patients positive by multiple-bead assay, 47.1% positive by multiple-bead assay and ELISA, and 95.2% positive by multiple-bead assay, ELISA, and ID had SSc. Although ID was not performed in all examined samples, this study indicates that multiple-bead assay can have a high rate of false-positive results (31).

In our cohort of patients with SSc-ILD, the anti-Scl-70 antibody assay performed by ID, but not CIA or LIA, had predictive significance for FVC decline after a year of follow-up (30). Moreover, among patients who were positive for anti-Scl-70 antibodies by LIA or CIA but negative by ID, the percentage of patients with a significant decline in FVC% was lower than that of those positive by ID and was similar to the percentage of patients who were negative for anti-Scl-70 antibodies by ID (see Table 3). This finding can have important implications for enrichment strategies in SSc-ILD clinical trials because it indicates that anti-Scl-70 antibody positivity as determined by LIA or CIA, contrary to ID, does not enrich the study population for fast progressors.

The present study has several strengths, including that this is the first study to compare ID, LIA, and CIA in SSc as well as their abilities to predict FVC% progression over time. Furthermore, this study was conducted in a well-characterized multiethnic cohort in which only patients with imaging-confirmed ILD were examined. However, the study also has some weaknesses. We could not evaluate the extent of ILD on HRCT as a predictor for disease progression because most HRCT studies were obtained in outside facilities and were not available for evaluation. Furthermore, the immunosuppressive regimens were heterogeneous given the observational nature of this sample. Thus, this study is not suitable for developing predictive biomarkers for a specific immunosuppressive treatment modality. Moreover, the investigated sample size was modest, and we cannot exclude that other variables will have predictive significance for ILD progression if a larger sample size is investigated. However, previous landmark SSc-ILD clinical trials had comparable sample sizes (7,8).

Our study underlines the differences observed between the various currently available anti-Scl-70 antibody assays. Given that this antibody is a widely used biomarker for SSc-associated ILD in both clinical and research settings, it calls for further refinement of the novel anti-Scl-70 antibody detection methods and the examination of their predictive significance for ILD progression.

In conclusion, anti-Scl-70 antibodies determined by ID was the only clinical variable that predicted faster FVC decline in patients with SSc-related ILD. Notably, both CIA and IB assay for the same antibody were not predictive of a higher rate of FVC% decline at their current cutoffs of positivity. The observed discrepancy between different methods of anti-Scl-70 antibody determination may have relevant implications for enrichment strategies in clinical trials of SSc-ILD as well as for patient stratification in clinical setting.

**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. Assassi 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.** Salazar, Hudson, Fritzler, Mayes, Assassi.

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