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Predictive Significance of Serum Interferon-Inducible Protein Score for Response to Treatment in Systemic Sclerosis–Related Interstitial Lung Disease

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Objective. Response to immunosuppression is highly variable in systemic sclerosis (SSc)–related interstitial lung disease (ILD). This study was undertaken to determine whether a composite serum interferon (IFN)–inducible protein score exhibits predictive significance for the response to immunosuppression in SSc-ILD.

Methods. Serum samples collected in the Scleroderma Lung Study II, a randomized controlled trial of mycophenolate mofetil (MMF) versus cyclophosphamide (CYC), were examined. Results were validated in an independent observational cohort receiving active treatment. A composite score of 6 IFN-inducible proteins IFNγ-inducible 10-kd protein, monokine induced by IFNγ, monocyte chemotactic protein 2, β_2-microglobulin, tumor necrosis factor receptor type II, and macrophage inflammatory protein 3β) was calculated, and its predictive significance for longitudinal forced vital capacity percent predicted measurements was evaluated.

Results. Higher baseline IFN-inducible protein score predicted better response over 3 to 12 months in the MMF arm (point estimate = 0.41, $P = 0.001$) and CYC arm (point estimate = 0.91, $P = 0.009$). In contrast, higher baseline C-reactive protein (CRP) levels were predictive of a worse ILD course in both treatment arms. The predictive significance of the IFN-inducible protein score and CRP levels remained after adjustment for baseline demographic and clinical predictors. During the second year of treatment, in which patients in the CYC arm were switched to placebo, a higher IFN-inducible protein score at 12 months showed a trend toward predicting a worse ILD course (point estimate = –0.61, $P = 0.068$), while it remained predictive of better response to active immunosuppression in the MMF arm (point estimate = 0.28, $P = 0.029$). The predictive significance of baseline IFN-inducible protein score was replicated in the independent cohort ($r_s = 0.43$, $P = 0.028$).

Conclusion. A higher IFN-inducible protein score in SSc-ILD is predictive of better response to immunosuppression and could potentially be used to identify patients who may derive the most benefit from MMF or CYC.

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INTRODUCTION

Interstitial lung disease (ILD) is the leading cause of disease-related mortality in systemic sclerosis (SSc) (1,2). Scleroderma Lung Study I (SLS I) (3) and SLS II (4) showed that both cyclophosphamide (CYC) and mycophenolate mofetil (MMF) were effective in the treatment of SSc-related ILD (SSc-ILD) as measured by serially obtained forced vital capacity percent predicted (FVC%) values. Moreover, a follow-up study indicated that short-term improvement in FVC% was associated with improved long-term survival (5). However, response to immunosuppression was highly variable between patients in both clinical trials. In addition, CYC and MMF can be associated with serious side effects (3,4,6). Ideally, their use should be reserved for the subset of patients who are likely to respond to these medications. However, there are no widely accepted clinical or biologic parameters to predict response to immunosuppression in SSc-ILD. Moreover, the extent of lung fibrosis on high-resolution computed tomography (HRCT) of the chest did not predict change in FVC% from baseline in patients treated with CYC in SLS I (3). Thus, there is a substantial unmet clinical need for novel predictive biomarkers in SSc-ILD.

The interferon (IFN) signature is the most prominent and robustly replicated gene expression signature in peripheral blood cells from SSc patients. This signature was first described in whole blood samples (7,8) but has since been replicated in peripheral blood mononuclear cells (9), as well as in lymphocytes and monocytes (10). Those studies indicated that approximately half of SSc patients have a "lupus-like" IFN gene expression signature in their peripheral blood cells (7). However, serum samples are more accessible during routine clinical care and a more practical source for biomarker development than peripheral blood cell RNA samples. Recent studies have shown that certain serum proteins correlate with the IFN gene expression signature in SSc (11,12), enabling the utilization of these serum proteins as surrogate markers for IFN activation status. The predictive significance of the IFN transcript or serum protein signature for response to immunosuppression has not been investigated in SSc.

Capitalizing on the valuable, prospectively collected serum samples in the SLS II study (4), we determined whether a composite serum IFN-inducible protein score has predictive significance for response to immunosuppression in SSc-ILD. We hypothesized that SSc patients with higher serum IFN-inducible protein levels would be more responsive to immunosuppressive therapy with either MMF or CYC.

PATIENTS AND METHODS

Study participants. All SLS II patients with an available baseline serum sample were included in the present study. The eligibility criteria for SLS II have been published previously (4). Briefly, key inclusion criteria were as follows: adults ages 18–75 years with well-defined SSc with limited or diffuse cutaneous involvement (13); active ILD as demonstrated by restrictive-to–borderline restrictive ventilatory impairment (FVC% <80–85 but ≥45) AND the presence of any ground-glass opacity on HRCT; exertional dyspnea (grade 2 or worse on the Magnitude of Task component of the Mahler Baseline Dyspnea Index [14]); and disease duration of <7 years (based on the first non–Raynaud's phenomenon symptom due to SSc). Key exclusion criteria included clinically significant pulmonary hypertension, clinically significant abnormalities on HRCT not attributable to SSc, smoking within the past 6 months, evidence of significant airflow obstruction, prior use of oral CYC or MMF for longer than 8 weeks, or use of CYC and/or MMF in the 30 days prior to randomization. The SLS II protocol was approved by the institutional review board of participating sites, and written informed consent was obtained from all study participants.

SLS II study design. Patients were randomized to receive either MMF for 2 years or oral CYC for 1 year followed by placebo for 1 year. Based on this design, patients in both treatment arms were receiving active treatment during the first 12 months, while the participants in the MMF arm were continued on MMF therapy and those in the CYC arm were placed on placebo during the second year. The FVC% was the primary outcome and was measured every 3 months during the 24-month study period. Serum protein levels were also measured in sera collected from 39 healthy controls at the University of Texas Health Science Center at Houston (UTHSC-H) (see Supplementary Methods, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract). SSc-related autoantibodies were determined at the UTHSC-H divisional laboratories, and the extent of disease based on involvement >20% was measured on HRCT (15,16) (see Supplementary Methods for more details).

Serum protein assays and calculation of the IFN-inducible protein score. Serum samples were collected at the baseline, 12-month, and 24-month visits and were immediately processed on-site on the day of collection according to a standardized protocol, and were subsequently aliquoted, stored in –80°C freezers, and shipped on dry ice in batches to the central biorepository at the UTHSC-H. All 133 participants (63 in the MMF arm and 70 in the CYC arm) with an available serum sample were included in the present study. Serum samples from healthy controls were processed and stored in the same manner as those from SLS II, except that no shipment was required. Only unthawed serum aliquots from SLS II participants and healthy controls were used.

The primary focus of the present study was the measurement of 6 IFN-inducible proteins: monokine induced by IFNγ (MIG), IFNγ-inducible 10-kd protein (IP-10), monocyte chemotactic protein 2 (MCP-2), β_2-microglobulin (β_2m), tumor necrosis factor receptor type II (TNFRII), and macrophage inflammatory protein 3β (MIP-3β). The corresponding gene names of these 6 proteins are

CXCL9, CXCL10, CCL8, B2M, TNFRSF1B, and CCL19, respectively. This protein list was selected following a 2-step process. In step 1, 14 serum cytokines were identified that correlated significantly ($r > 0.3$ and false discovery rate–adjusted $P < 0.05$) with the IFN gene expression signature in the baseline samples collected in the Scleroderma: Cyclophosphamide or Transplantation (SCOT) study (see Supplementary Material in ref. 12). In step 2, 6 of these proteins were also confirmed as inducible by type I IFN in human peripheral blood cells based on in vitro studies, according to the information obtained from the Interferome V2.0 database (17).

Serum protein assays were performed at the Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory of Myriad Rules-Based Medicine using multianalyte profiling (MAP) multiplexed immune assay. Although the primary focus of the present study was IFN-inducible proteins, these serum proteins could not be measured in isolation with predesigned multiplex panels. Therefore, 57 other serum proteins belonging to predesigned Myriad MAPs were also measured as part of the multiplex assay. For the analysis, proteins with levels below the lower limit of quantification in >50% of the baseline SLS II participants were excluded. For the remainder of the proteins, levels below the lower limit of quantification were replaced by the lower limit of quantification, while levels above the upper limit of quantitation were replaced by the upper limit of quantitation. The 6 IFN-inducible proteins listed above were within the dynamic range of their respective assays for all samples and no adjustments were necessary. Thirty-four of the other 57 proteins, including high-sensitivity C-reactive protein (CRP), were detectable in >50% of baseline SLS II samples and were further analyzed. In addition, Simoa assays (Quanterix) (18) were used for ultrasensitive detection of 2 low-abundant cytokines, B lymphocyte chemoattractant (CXCL13) and interleukin-6 (IL-6), which have previously been implicated as biomarkers in SSc-ILD (19,20).

A composite score of MIG, IP-10, MCP-2, β_2m, TNFRII, and MIP-3β was calculated using a previously described method (7,11,21–23). Specifically, the protein levels were divided by the top 95th percentile for each protein. Next, all values in the top 5% category were assigned a value of 1.0. Finally, the normalized values for the 6 proteins were summed to obtain the IFN-inducible protein score.

Confirmation cohort. For independent confirmation of the study results, patients with SSc enrolled in the Prospective Registry for Early Systemic Sclerosis (PRESS) cohort were evaluated. Briefly, PRESS is a multicenter, observational cohort of patients with early diffuse cutaneous SSc (disease duration <3 years from onset of the first non–Raynaud's phenomenon symptom of SSc) (24). All enrolled patients who fulfilled the following criteria were included in the present study: available serum sample at the baseline visit, no missing FVC% data at the baseline and 12-month visits, evidence consistent with SSc-ILD on HRCT, and treatment with immunosuppressive agents during the first year of the follow-up period. The serum samples in PRESS were processed and stored following the same procedures as in SLS II. Moreover, levels of IFN-inducible proteins and CRP were measured using the same assays in the Myriad Rules-Based Medicine laboratory.

Statistical analysis. Depending on the distribution, raw or $\log_2$-transformed cytokine data were analyzed. Similar to the primary clinical outcome analysis in SLS II (4), a joint model (25) combining a mixed-effects model for the longitudinally obtained FVC% values with a survival model to handle non-ignorable missing data due to study dropouts, treatment failure, or death was used. In the primary analysis, the outcome was the course of FVC% measured at 3-month increments from month 3 to month 12, which corresponds to the time period in which patients in both treatment arms were receiving active treatment. The longitudinal model in the primary analysis included the following covariates: baseline protein level, baseline FVC%, and a linear time trend. In addition, an extended multivariable analysis was performed that contained baseline protein levels (i.e., IFN-inducible protein score and CRP), in addition to baseline demographic and clinical variables that showed predictive significance in separate analyses ($P < 0.05$), baseline FVC%, and a linear time trend.

In a secondary analysis, we also investigated whether the serum protein levels at the 12-month visit had predictive significance for the course of FVC% over the 15-month to 24-month visits. The longitudinal model in this analysis included the following covariates: protein levels at the 12-month visit, FVC% at the 12-month visit, and linear splines with a knot at 21 months to characterize the time trend. The *P* value for the analysis of individual serum protein levels was adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (26).

In the confirmation cohort (PRESS), the majority of patients evaluated had only 2 FVC% measurements available during the first 12 months after enrollment (baseline and 12-month visit), thus a different, simplified approach for the analysis of data from these 2 time points was used. As previously described (27), the predictive significance of IFN-inducible protein score for percent change in FVC% ($[FVC\%_{\text{12-month visit}} - FVC\%_{\text{baseline}}]/FVC\%_{\text{baseline}}$) was analyzed by Spearman's correlation.

All tests were 2-sided. The joint analyses were performed using the R package JMbayes, and all other analyses were conducted in SAS version 9.4 (SAS Institute).

RESULTS

Baseline characteristics of the participants. Of the 142 patients enrolled, serum samples were available for 133 patients at baseline, 99 patients at the 12-month visit, and 84 patients at the 24-month visit. The healthy controls were similar to SLS II participants with regard to age, sex, and ethnic background (see Supplementary Table 1, available on the *Arthritis & Rheumatology* website

at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract for patient and control characteristics).

IFN-inducible protein score in patients and controls. The SLS II participants had a significantly higher IFN-inducible protein score at the baseline visit than healthy controls (fold difference 2.19; $P < 0.001$). As shown in Supplementary Figure 1 (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract), the IFN-inducible protein score decreased significantly from the baseline visit to the 12-month visit (fold change 0.75; $P < 0.001$ for the MMF arm and fold change 0.76; $P < 0.001$ for CYC arm). In the subgroup of patients with serum samples available at both the 12-month and 24-month visits (n = 43 in the MMF arm and n = 41 in the CYC arm), the IFN-inducible protein score did not change significantly from the 12-month visit to the 24-month visit ($P = 0.994$ for MMF and $P = 0.529$ for CYC) (Supplementary Figure 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract). As shown in Supplementary Table 2 (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract), the baseline demographic and clinical variables did not show a significant association/correlation with the concurrent IFN-inducible protein score.

Predictive significance of individual serum protein levels for ILD course. As described above, serum levels of 6 IFN-inducible proteins, as well as 36 serum proteins involved in other immune pathways, were measured in the baseline SLS II samples as part of the multiplex assay. We subsequently investigated whether any individual baseline protein levels had predictive significance for the course of FVC% from month 3 to month 12 of the follow-up period. As shown in Supplementary Table 3 (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract), only 2 serum proteins, MIG and IP-10 (both IFN-inducible proteins) showed predictive significance for FVC% in both treatment arms in the same direction after correction for multiple comparisons. Specifically, higher baseline MIG and IP-10 levels predicted higher serial FVC% levels. The point estimates for the other 4 IFN-inducible proteins were also toward higher serial FVC% levels, although their associations did not reach statistical significance. Of note, 2 other proteins (intercellular adhesion molecule 1 and eotaxin 1) also reached statistical significance in both treatment arms after correction for multiple comparisons, but the direction of prediction was not consistent between the 2 SLS II treatment arms for these 2 proteins.

Predictive significance of IFN-inducible protein score for ILD course. Next, the predictive significance of the IFN-inducible protein score was investigated. As shown in Table 1, a higher baseline IFN-inducible protein score predicted better ILD course based on higher serial FVC% values from month 3 to month 12 in both treatment arms after adjustment for baseline FVC% (point estimate 0.41, $P = 0.001$ for MMF and point estimate 0.91, $P = 0.009$ for CYC).

In the secondary analysis pertaining to the second year of SLS II, during which patients in the MMF arm continued to receive MMF and those in the CYC arm were switched to placebo (Table 1), higher IFN-inducible protein scores at 12 months continued to predict better response to immunosuppression in the MMF arm (point estimate 0.28, $P = 0.029$), while higher IFN-inducible protein scores at 12 months showed a trend toward predicting

Table 1. Predictive significance of IFN-inducible protein score for subsequent serial FVC% values in patients with SSc-ILD treated with MMF or CYC*

	Point estimate (95% CI)	*P*
MMF arm		
Predictive significance of baseline IFN-inducible protein score for serial FVC% values from month 3 to month 12		
Baseline IFN-inducible protein score	0.41 (0.23, 0.59)	0.001
Baseline FVC%	0.84 (0.82, 0.86)	<0.001
Predictive significance of 12-month IFN-inducible protein score for serial FVC% values from month 15 to month 24		
12-month IFN-inducible protein score	0.28 (0.11, 0.69)	0.029
12-month FVC%	0.96 (0.9, 0.98)	<0.001
CYC arm		
Predictive significance of baseline IFN-inducible protein score for serial FVC% values from month 3 to month 12		
Baseline IFN-inducible protein score	0.91 (0.56, 1.13)	0.009
Baseline FVC%	0.87 (0.84, 0.9)	<0.001
Predictive significance of 12-month IFN-inducible protein score for serial FVC% values from month 15 to month 24		
12-month IFN-inducible protein score	−0.61 (−1.5, 0.11)	0.068
12-month FVC%	1 (0.96, 1.08)	<0.001

* All models included time as an independent variable. IFN = interferon; FVC% = forced vital capacity percent predicted; SSc-ILD = systemic sclerosis–related interstitial lung disease; MMF = mycophenolate mofetil; CYC = cyclophosphamide; 95% CI = 95% confidence interval.

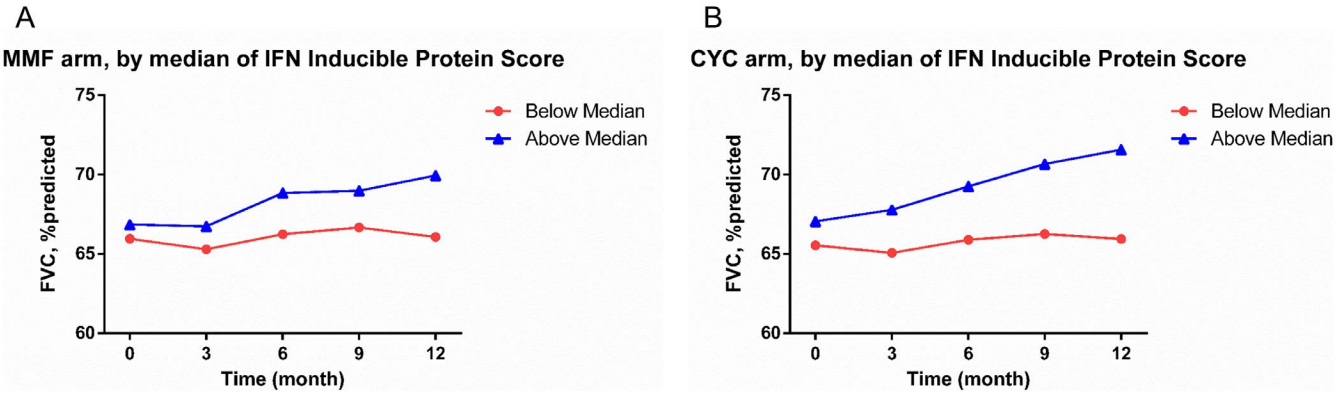

Figure 1. Course of forced vital capacity percent predicted (FVC%) from randomization to 12 months, according to interferon (IFN)–inducible protein score, in patients with systemic sclerosis (SSc)–related interstitial lung disease (ILD) treated with mycophenolate mofetil (MMF) (**A**) or cyclophosphamide (CYC) (**B**). IFN-inducible protein score was dichotomized using the median value. Patients with a positive IFN-inducible protein score (higher than the median value) had higher FVC% levels in the both the MMF arm ($P = 0.003$) and the CYC arm ($P = 0.004$). The analysis was adjusted for baseline FVC% and, similar to Tables 1–3, baseline FVC% and time were added as independent variables to the joint model.

lower serial FVC% values from month 15 to month 24 during the placebo treatment period in the CYC arm (point estimate = –0.61; $P = 0.068$).

Our previous gene expression studies have shown that approximately half of patients with SSc have an IFN signature (7,12). Building on this finding, the IFN-inducible protein score was dichotomized based on the median value in the baseline patient samples. As shown in Figure 1, patients with a positive baseline IFN-inducible protein score had a more favorable ILD course from month 3 to month 12 in both treatment arms compared with patients with a negative IFN-inducible protein score (point estimate 1.28, $P = 0.003$ for MMF and point estimate 2.6, $P = 0.004$ for CYC).

We also examined whether the baseline IFN-inducible protein score had predictive significance for the course of diffusing capacity for carbon monoxide percent predicted (DLco%) from month 3 to month 12 after randomization. Consistent with the FVC% findings, higher IFN-inducible protein score predicted higher serial DLco% in the CYC arm (point estimate 0.7 [95% confidence interval 0.47, 0.96]; $P < 0.001$). However, IFN-inducible protein score did not significantly predict DLco% course in the MMF arm (point estimate –0.15 [95% confidence interval –0.42, 0.15]; $P = 0.146$).

Table 2. Predictive significance of CRP for subsequent serial FVC% values in patients with SSc-ILD treated with MMF or CYC*

	Point estimate (95% CI)	*P*
MMF arm		
Predictive significance of baseline CRP for serial FVC% values from month 3 to month 12		
Baseline CRP†	−0.15 (−0.31, −0.01)	0.038
Baseline FVC%	0.83 (0.78, 0.86)	<0.001
Predictive significance of 12-month CRP for serial FVC% values from month 15 to month 24		
12-month CRP†	−0.61 (−0.7, −0.51)	<0.001
12-month FVC%	0.98 (0.96, 0.99)	<0.001
CYC arm		
Predictive significance of baseline CRP for serial FVC% values from month 3 to month 12		
Baseline CRP†	−0.56 (−0.72, −0.45)	<0.001
Baseline FVC%	0.90 (0.86, 0.92)	<0.001
Predictive significance of 12-month CRP for serial FVC% values from month 15 to month 24		
12-month CRP†	−0.3 (−0.93, −0.08)	0.027
12-month FVC%	1.01 (0.97, 1.12)	<0.001

* All models included time as an independent variable. CRP = C-reactive protein (see Table 1 for other definitions).
† Log_2 transformed.

Table 3. Separate analyses to examine the predictive significance of baseline demographic and clinical variables for serial FVC% values from month 3 to month 12 in patients with SSc-ILD treated with MMF or CYC*

	MMF arm		CYC arm	
Baseline variable	Point estimate (95% CI)	*P*	Point estimate (95% CI)	*P*
Age in years	−0.05 (−0.18, 0.08)	0.462	0.04 (−0.06, 0.15)	0.411
Female sex	0.04 (−0.53, 0.69)	0.891	1.17 (−0.09, 2.26)	0.058
African American race	−0.68 (−1.26, −0.13)	0.032†	−2.4 (−3.04, −1.9)	<0.001†
Diffuse disease type	1.15 (0.43, 2.06)	0.005†	−1.97 (−3.34, −0.77)	0.008†
Disease duration	0.04 (−0.06, 0.15)	0.314	0.12 (0.01, 0.25)	0.042†
MRSS	0.07 (0.04, 0.11)	0.002†	−0.04 (−0.14, 0.06)	0.392
Antitopoisomerase	−0.14 (−1.12, 0.81)	0.729	−0.35 (−2.23, 1.62)	0.654
Anti–RNA polymerase	0.83 (−0.61, 2.06)	0.175	1.08 (−2.08, 4.17)	0.425
Extensive disease on HRCT‡	−2.45 (−2.85, −2.11)	<0.001†	0.09 (−2.18, 2.36)	0.79

* Each row represents a separate model that included one baseline clinical variable, baseline FVC%, and time as independent variables. MRSS = modified Rodnan skin thickness score (see Table 1 for other definitions).
† Baseline demographic and clinical variables showing predictive significance in separate models that were included in the subsequent extended multivariable model (see Tables 4 and 5).
‡ Quantitative ILD >20% on high-resolution computed tomography (HRCT) of the chest.

Predictive significance of CRP level for ILD course. Contrary to the favorable (i.e., positive) predictive value of the IFN-inducible protein score, higher CRP levels predicted a worse ILD course reflected in lower serial FVC% values from month 3 to month 12 in both treatment arms after adjustment for baseline FVC% (Table 2). In the secondary analysis, higher CRP levels at 12 months again predicted a worse ILD course reflected by lower serial FVC% values from month 15 to month 24 in both treatment arms (Table 2).

IFN-inducible protein score and CRP level are independent predictors of ILD course. As shown in Table 3, the predictive significance of baseline demographic and clinical variables for serial FVC% values from month 3 to month 12 were first examined in separate models after adjustment for baseline FVC% for each treatment arm. Next, the predictive significance of the IFN-inducible protein score and CRP level (both as continuous variables) was investigated in an extended multivariable model after adjustment for baseline FVC%, in addition to variables showing predictive significance in the separate analyses described above, in the MMF arm (Table 4) and in the CYC arm (Table 5). Similar to the findings described above, higher baseline IFN-inducible protein scores predicted better ILD course, and higher baseline CRP levels predicted worse ILD course, from month 3 to month 12 after adjustment for baseline demographic and clinical variables in both treatment arms.

Table 4. Predictive significance of baseline IFN-inducible protein score and CRP level, after adjustment for baseline demographic and clinical variables, for serial FVC% values from month 3 to month 12 in patients with SSc-ILD treated with MMF*

Baseline variable	Point estimate (95% CI)	*P*
IFN-inducible protein score	0.32 (0.11, 0.52)	0.013
CRP†	−0.13 (−0.24, −0.01)	0.041
African American race	0.95 (0.43, 1.41)	0.004
Diffuse disease type	0.39 (−0.19, 1.05)	0.139
MRSS	0.05 (0.03, 0.09)	0.008
Baseline FVC%	0.81 (0.78, 0.83)	<0.001
Extensive disease on HRCT‡	−2.27 (−2.70, −1.80)	<0.001

* Time was included as an independent variable. CRP = C-reactive protein; MRSS = modified Rodnan skin thickness score (see Table 1 for other definitions).
† Log_2 transformed.
‡ Quantitative ILD >20% on high-resolution computed tomography (HRCT) of the chest.

Confirmation of the predictive significance of the IFN-inducible protein score in an independent cohort. The predictive significance of the IFN-inducible protein score and CRP level was investigated in the independent, observational PRESS cohort. In this cohort, 47 patients had a baseline serum sample and had FVC% measurements at the baseline and 12-month visits; of these, 31 (66%) had evidence of SSc-ILD on HRCT. Of these 31 patients, 26 were treated with immunosuppressive agents (23 with MMF and 3 with methotrexate) during the first year of the follow-up period and were included in the present study.

Table 5. Predictive significance of baseline IFN-inducible protein score and CRP level, after adjustment for baseline demographic and clinical variables, for serial FVC% values from month 3 to month 12 in patients with SSc-ILD treated with CYC*

Baseline variable	Point estimate (95% CI)	*P*
IFN-inducible protein score	0.92 (0.79, 1.04)	<0.001
CRP†	−0.46 (−0.53, −0.39)	<0.001
African American race	−2.01 (−2.31, −1.71)	<0.001
Diffuse disease type	−0.60 (−0.91, −0.33)	0.005
Disease duration	0.19 (0.12, 0.26)	0.002
Baseline FVC%	0.90 (0.89, 0.91)	<0.001

* Time was included as an independent variable. CRP = C-reactive protein (see Table 1 for other definitions).
† Log_2 transformed.

Supplementary Table 4, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41627/abstract, shows their demographic and clinical characteristics. Confirming our findings in SLS II, higher baseline IFN-inducible protein score predicted increasing FVC% values; specifically, the baseline IFN-inducible protein levels correlated positively with percent change in FVC% at 12 months (Spearman's correlation coefficient [r_s] = 0.43, P = 0.028). This correlation remained significant even after exclusion of the 3 patients treated with methotrexate (r_s = 0.47, P = 0.023) (n = 23). Of note, baseline CRP level was not predictive of percent change in FVC% at 12 months in the PRESS cohort (P = 0.828).

DISCUSSION

In the well-characterized SLS II clinical trial cohort, a higher IFN-inducible protein score predicted better response to MMF, as well as CYC, while higher baseline CRP levels predicted a worse ILD course. Moreover, the predictive significance of the IFN-inducible protein score was independent of CRP level and clinical/demographic predictors. In the validation analysis, the predictive significance of the IFN-inducible protein score was confirmed in the PRESS cohort of patients with early diffuse cutaneous SSc.

In this study, a rigorous method was employed for calculation of the serum IFN-inducible protein score. Specifically, serum proteins included in the IFN-inducible protein score correlated with the peripheral blood cell IFN transcript signature in our previous study of untreated SSc patients using the same protein assays (12) and were induced by type I IFN in in vitro studies of human peripheral blood cells. Moreover, the method used for calculation of the composite score weighted each protein equally (21–23), ensuring that the overall IFN-inducible protein score is not skewed by a few outlier values of 1 or 2 proteins. Thus, the IFN-inducible protein score used in this study provides an accurate reflection of the type I IFN activation status in circulation in SSc-ILD. Of note, there is substantial overlap between type I and type II IFN-inducible genes/proteins. Based on the information in the Interferome database, the 6 serum proteins utilized can be induced by both type I and type II IFN. Therefore, we cannot exclude the possibility that the IFN composite score evaluated in this study is in part driven by type II IFN. However, in a pilot study of anifrolumab (a blocking antibody against IFNAR1) in 26 SSc patients, 2 of the proteins included in the composite score (β_2m and IP-10 [CXCL10]), decreased significantly after blocking the type I IFN receptor (28), providing direct human evidence that the IFN-inducible protein score is at least in part driven by type I IFN in patients with SSc.

In the present study, SSc-ILD patients with a higher IFN-inducible protein score were more responsive to immunosuppression with CYC or MMF. However, the results from the second year of the CYC arm (placebo phase) indicated that patients with an IFN excess profile at the 12-month visit had a worse ILD course without concurrent immunosuppressive treatment, while higher IFN-inducible protein score at the same visit continued to be predictive of better ILD course in patients assigned to the MMF arm, who continued to receive active immunosuppressive treatment during the second year of the study. This finding supports the notion that a high IFN score adversely affects SSc-ILD progression unless immunosuppressive treatment is administered. Thus, the IFN-inducible protein score in SSc acts as a predictive biomarker identifying likely responders to treatment rather than a prognostic biomarker that predicts the natural history of disease regardless of treatment status.

The deleterious effect of IFN excess in SSc is supported by previous murine model and human studies (for review, see ref. 29). In a previous study on the role of IFN regulatory factor 5 (IRF-5), bleomycin-induced dermal and lung fibrosis was attenuated in IRF-5–deficient mice. Moreover, there was in vitro evidence that profibrotic transcriptional activity of IRF-5 in fibroblasts was enhanced by transforming growth factor β (TGFβ) (30). In a more recent study on the role of IRF-7 in SSc pathogenesis, bleomycin-induced dermal fibrosis, as well as hypodermal fibrosis in tight skin mice, was attenuated in IRF-7–deficient mice. Moreover, IRF-7 blockade attenuated fibrotic response to TGFβ in SSc dermal fibroblasts (31). In terms of direct human data, a previous randomized controlled trial in which SSc patients were treated with recombinant IFNα or placebo had to be stopped prematurely because IFNα-treated patients demonstrated a significantly worse ILD course as measured by FVC% (32). More recently, in a phase I trial of the anti–type I IFN receptor antibody anifrolumab for the treatment of SSc, skin gene expression studies showed evidence of suppressed TGFβ signaling in the anifrolumab-treated group (28). Taken together, these data indicate that IFN excess is deleterious in SSc but also identifies patients who are more likely to benefit from immunosuppressive treatment.

In the present study, higher CRP level predicted worse ILD course in SSc patients receiving active immunosuppressive treatment as well as during the placebo phase in the CYC arm in the second year of the study, indicating that CRP, as a general marker of inflammation, is a prognostic biomarker that predicts worse FVC course regardless of treatment status. This finding is also supported by previous observational studies showing that higher baseline CRP levels are predictive of reduced survival (33) and faster FVC% decline in SSc (34). More recently, in a retrospective study of 24 SSc-ILD patients treated with 6 monthly infusions of CYC, a higher CRP level was significantly associated with poor response (35). Of note, higher CRP levels in the confirmation cohort did not predict the course of ILD in the present study. This might be due to the small sample size and/or the more heterogeneous patient population in the PRESS cohort, where a general marker of inflammation like CRP can be influenced by extrapulmonary factors. Moreover, patients in the PRESS cohort had different baseline characteristics than SLS II participants. Specifically, all PRESS patients had diffuse cutaneous involvement and had a disease duration of <3 years. Moreover, 30% of the

PRESS patients evaluated had baseline FVC% >85% and therefore would have not met one of the inclusion criteria for SLS II.

In addition to IFN-inducible proteins, levels of 36 immune pathway–related serum proteins, including IL-6, were measured in the present study. In a previous observational study, higher IL-6 levels were predictive of worse ILD course (19). In the present study, IL-6 levels were not predictive of FVC% course in either the MMF or the CYC arm (Supplementary Table 3). Similarly, anti–topoisomerase I was not predictive of ILD course (Table 3). This finding is not consistent with our previous finding in an observational cohort of early SSc patients with or without ILD, in which anti–topoisomerase I was predictive of a faster decline in FVC% (36), supporting the notion that antitopoisomerase loses its predictive significance in a study population that includes only patients with clinically significant ILD.

This study has several strengths. To our knowledge, this is the first study examining the predictive role of serum IFN-inducible proteins in a randomized controlled clinical trial of SSc-ILD. All serum protein assays were performed in the same CLIA-certified laboratory using rigorously standardized procedures. In SLS II, repeated FVC% measurements were available, allowing for a more accurate reflection of ILD progression. Patients were treated according to standardized, uniform treatment protocols, decreasing the potential confounding effect of treatment heterogeneity. Moreover, the predictive significance of the IFN-inducible protein score was shown in both the MMF and CYC arms separately, and confirmed in an independent observational study. Finally, SLS II was conducted in 14 centers across the US and included patients from a diverse ethnic background, increasing the generalizability of our findings.

There were several limitations to the present study. The sample size in the confirmation cohort was relatively small. Furthermore, SLS II did not include a placebo arm during the first year of the study period, although this limitation is partially mitigated by the fact that the IFN-inducible protein score showed prediction in opposite directions during the second year when patients in the CYC arm were switched to placebo while patients in the MMF arm continued receiving active immunosuppressive treatment. Furthermore, SLS II only included patients with a disease duration of <7 years; therefore, we could not investigate the predictive significance of the IFN-inducible protein score in patients with longstanding disease. Moreover, our findings should be further investigated in the recently completed study of nintedanib treatment for SSc-ILD (37) and future large clinical trials of antifibrotic agents in SSc-ILD, with the ultimate goal of developing prediction models for identifying patients who would primarily benefit from immunosuppressive versus antifibrotic treatment.

In conclusion, SSc-ILD patients with a higher serum IFN-inducible protein score are more likely to respond to MMF or CYC. The predictive significance of IFN-inducible protein score is independent of the general marker of inflammation CRP, which predicted worse ILD course regardless of the treatment regimen in SLS II. These serum proteins may be useful for more informed clinical decisions and clinical trial design and may ultimately lead to more personalized treatment regimens in SSc-ILD.

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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. Assassi, Li, Volkmann, Roth, Hinchcliff, Khanna, Frech, Steen, Elashoff, Tashkin.

Acquisition of data. Assassi, Volkmann, Mayes, Roth, Hinchcliff, Khanna, Frech, Clements, Furst, Goldin, Bernstein, Castelino, Domsic, Gordon, Hant, Shah, Shanmugam, Steen, Tashkin.

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