Correlation of Interferon-Inducible Chemokine Plasma Levels With Disease Severity in Systemic Sclerosis


Objective. To measure interferon (IFN)–inducible chemokines in the plasma of patients with systemic sclerosis (SSc) and investigate whether the chemokine levels are correlated with disease severity.

Methods. Plasma levels of the IFN-inducible chemokines IFN-γ-inducible protein 10 (IP-10/CXCL10), IFN-inducible T cell α chemoattractant (I-TAC/CXCL11), and monocyte chemoattractant protein 1 (CCL2) were measured in SSc patients and examined for correlation with the IFN gene expression signature. A composite IFN-inducible chemokine score was generated for chemokines showing a correlation with the IFN gene signature (IP-10 and I-TAC), and this score was compared between 266 patients with SSc enrolled in the Genetics versus Environment in Scleroderma Outcome Study (GENISOS) cohort and 97 matched control subjects. Subsequently, the correlation between the IFN-inducible chemokine score at baseline and markers of disease severity was assessed. In addition, the course of the IFN-inducible chemokine score over time was examined.

Results. The plasma IFN-inducible chemokine score correlated with the IFN gene expression signature, and this score was higher in SSc patients compared to controls. The IFN-inducible chemokine score was also associated with the absence of anti–RNA polymerase III antibodies and presence of anti–U1 RNP antibodies, but not with disease duration, disease type, or other autoantibodies. The chemokine score correlated with concomitantly obtained scores on the Medsger Severity Index for muscle, skin, and lung involvement in SSc, as well as the forced vital capacity, diffusing capacity for carbon monoxide, and creatine kinase levels. The association of the chemokine score with disease severity was independent of the presence of anti–U1 RNP or other potential confounders (age, sex, ethnicity, disease duration, and treatment with immunosuppressive agents). Finally, there was not a significant change in the IFN-inducible chemokine score over time.

Conclusion. The IFN-inducible chemokine score is a stable serologic marker of a more severe form of SSc and may be useful for risk stratification of patients, regardless of disease type (limited or diffuse) or duration of disease.

Immune dysregulation has been proposed as an important contributor to the pathogenesis of systemic sclerosis (SSc; scleroderma). Several groups of investigators have reported that peripheral blood cells from patients with SSc demonstrate dysregulation of interferon (IFN)–inducible gene expression (1–5). In a previous large, global gene expression study, we have demonstrated that the IFN gene signature in patients with SSc is similar to that seen in patients with systemic lupus erythematosus (SLE) (6). Further studies have shown that in patients with SLE, the composite score for serum
chemokine levels is tightly correlated with the IFN gene expression signature (7,8). In a small study of 30 patients with SLE, this serum chemokine composite score also showed a higher correlation with disease activity measures than with the IFN gene expression score (7). Similar observations have been made in patients with dermatomyositis (9). Of note, although these chemokines can be induced by IFN, it is likely that the IFN-inducible chemokines are not regulated exclusively by either type I or type II IFN.

The course of SSc is highly variable, ranging from stable, mild involvement to progressive disease leading to widespread fibrosis of the skin and internal organs. Currently available demographic and clinical parameters are not sufficient for predicting the course of the disease in patients with varying degrees of disease severity.

In this study, we hypothesized that in patients with SSc, plasma levels of IFN-inducible chemokines are correlated with the IFN gene signature and are associated with disease severity. We first examined the correlation of IFN-γ-inducible protein 10 (IP-10/CXCL10), IFN-inducible T cell α chemoattractant (I-TAC/CXCL11), and monocyte chemoattractant protein 1 (MCP-1/CCL2) with the IFN gene signature, because these chemokines have been shown to correlate with the IFN gene signature and with disease severity in patients with SLE (7,8) and patients with dermatomyositis (9). We then investigated the association of our IFN-inducible chemokine score with clinical features of SSc at the cross-sectional level. Finally, the longitudinal changes in IFN-inducible chemokine levels and their correlation with clinical disease parameters were examined.

PATIENTS AND METHODS

Study participants. All patients with SSc met the American College of Rheumatology 1980 preliminary criteria for the classification of SSc (10) or had 3 of 5 signs of CREST syndrome (calcinosis, Raynaud’s phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias, with the presence of sclerodactyly being mandatory). The selection criteria for the study subjects, used for calculation of the IFN gene expression signature, were described in our previous report (6). For measurements of plasma chemokine levels and chemokine–clinical data correlations, we recruited SSc patients from the prospective Genetics versus Environment in Scleroderma Outcome Study (GENISOS) (n = 266). Control subjects (n = 97) were individuals who had no history of autoimmune diseases and were matched by age, sex, and ethnicity to the patients with SSc. In addition to the plasma samples collected from all SSc patients at baseline, a followup plasma sample was available from 63 of the patients.

All study subjects provided written informed consent to participate. The study was approved by the Institutional Review Boards of all participating centers.

Plasma chemokine measurements and calculation of the IFN-inducible chemokine score. We measured chemokine levels in the plasma of patients enrolled in the GENISOS cohort and the plasma of unaffected control subjects. These samples were collected using EDTA blood collection tubes, and stored at −80°C until analyzed. The plasma had not undergone more than 2 freeze–thaw cycles before the chemokine levels were determined.

Chemokine levels were measured by enzyme-linked immunosorbent assay (ELISA), utilizing electrochemiluminescent multiplex assays (Meso Scale Discovery) (11). Each sample was run in duplicate. The plasma levels of IP-10 and I-TAC were determined in all 266 samples collected from SSc patients at baseline and in the 63 followup samples from SSc patients, while levels of MCP-1 were measured only in a subgroup of patients with concomitant microarray/quantitative polymerase chain reaction (qPCR) data. The relative levels of IP-10 and I-TAC were used for calculation of the IFN-inducible chemokine score, following a normalization method by Bauer et al (7). Specifically, concentration values were normalized according to the 95th percentile of plasma chemokine levels across all samples, and the normalized values were summed up to obtain the IFN-inducible chemokine score.

Calculation of the IFN gene expression signature. We calculated the microarray IFN gene expression score based on the levels of the 43 IFN-inducible transcripts that were differentially expressed in patients with SSc when compared to unaffected controls in our previous global gene expression study (6). The cumulative microarray IFN gene expression score was calculated as previously described (6,12).

We also calculated the qPCR IFN gene expression score by calculating a composite score of relative transcript levels of 3 IFN-inducible genes (STAT1, IFI6, and IFIT3). The transcript levels of these 3 genes were determined by qPCR analysis, as previously described (6). Of note, some of the genes included in the microarray and qPCR scores, such as STAT1, can be induced by both type I and type II IFNs.

Clinical outcome measures. The disease duration was calculated using 2 different starting points, defined as follows: 1) The onset of disease from the first non–Raynaud’s phenomenon symptom; or 2) The onset of disease from the first symptom (Raynaud’s phenomenon or non–Raynaud’s phenomenon) attributable to SSc. Autoantibodies, including anti–RNA polymerase III (anti–RNAP III) antibodies, anti-U1 RNP antibodies, anticentromere antibodies (ACAs), and antitopoisoasemerase antibodies, were detected in all SSc serum samples at the laboratories of the Division of Rheumatology of University of Texas Health Science Center at Houston. Briefly, antinuclear antibodies and ACAs were detected by indirect immunofluorescence using HEp-2 cell substrates (Antibodies Inc.). Antitopoisoasemerase, anti-Ro, and anti–U1 RNP antibodies were determined by passive immunodiffusion against calf thymus extract (Inova Diagnostics). Testing for anti–RNAP III antibodies was performed using an ELISA (MBL).

For the analyses of association with SSc-related antibodies, we excluded patients with more than 1 SSc-related antibody (n = 7) and patients with anti-Ro positivity (n = 10). The anti-Ro–positive patients were excluded because these
antibodies are not specific to SSc and have been linked to the IFN-inducible gene expression signature (6).

The Medsger Severity Index for components of SSc, which measures the clinical severity of disease in several affected areas, including the skin, muscle, gastrointestinal tract, lung, heart, and kidney, was determined prospectively in all patients (13,14). Furthermore, other clinical data were concomitantly collected from each patient, including the per-cent predicted forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO), the modified Rodnan skin thickness score (MRSS) (15), and the creatine kinase level; these measures were used as additional surrogates for the severity of interstitial lung disease (ILD) (16), skin involvement, and muscle involvement, respectively. Medication information was also collected prospectively. Patients who were taking immunosuppressive agents (with the exception of those receiving hydroxychloroquine or a dosage of ≤5 mg per day prednisone or equivalent) at the time of blood withdrawal were categorized as being treated with immunosuppressive agents. A complete blood cell count was also performed concomitantly in all plasma samples at baseline.

**Statistical analysis.** We first examined the correlation of the IFN-inducible chemokine score with the microarray IFN gene expression score and qPCR IFN gene expression score by Spearman’s rank order test (Spearman’s rho). A nonparametric approach was chosen for this analysis, due to the small sample size (n = 24 patients). Subsequently, we used Student’s t-test to compare the log-transformed chemokine levels between 266 SSc patients enrolled in the GENISOS cohort and 97 unaffected controls. We did not observe significant deviations from normal distribution for any of the log-transformed plasma chemokine levels, as determined by a D’Agostino test for normality. The associations of log-transformed plasma chemokine levels with the clinical manifestations of SSc were investigated by univariable linear regression and Pearson’s correlation analysis (Pearson’s r).

To adjust for potential confounders, multivariable linear regression models were constructed. First, the association of disease subtypes (independent variable) with chemokine levels (outcome variable) was examined after adjustment for potential confounding demographic variables and treatment with immunosuppressive agents. Subsequently, the correlation of chemokine levels (independent variable) with disease severity (outcome variable) was examined after adjustment for potential demographic confounders, disease duration, and treatment with immunosuppressive agents. The first-order interaction term between the treatment status with immunosuppressive agents and the investigated independent variables

Table 1. Correlations of plasma levels of interferon (IFN)–inducible chemokines with the IFN gene expression signature in patients with systemic sclerosis*

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Correlation with microarray IFN score</th>
<th>Correlation with qPCR IFN score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Pcorr</td>
</tr>
<tr>
<td>IP-10</td>
<td>0.007</td>
<td>0.021</td>
</tr>
<tr>
<td>I-TAC</td>
<td>0.010</td>
<td>0.030</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.27</td>
<td>0.81</td>
</tr>
<tr>
<td>IP-10/I-TAC</td>
<td>0.0015</td>
<td>0.0045</td>
</tr>
<tr>
<td>IP-10/I-TAC/MCP-1</td>
<td>0.0198</td>
<td>0.0594</td>
</tr>
</tbody>
</table>

* Correlations of chemokine levels with the IFN gene expression signature, determined by microarray or quantitative polymerase chain reaction (qPCR), were assessed by Spearman’s correlation coefficient (rho). \( P_{corr} = P \) value corrected for multiple comparisons; IP-10 = IFN-γ-inducible protein 10; I-TAC = IFN-inducible T cell α chemoattractant; MCP-1 = monocyte chemoattractant protein 1.

Table 2. Characteristics of the patients with systemic sclerosis (SSc) in the Genetics versus Environment in Scleroderma Outcome Study (GENISOS) cohort (n = 266) and unaffected control subjects (n = 97)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GENISOS SSc cohort</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>221 (83)</td>
<td>78 (80)</td>
</tr>
<tr>
<td>Age at the time of first study visit, mean ± SD years</td>
<td>48.6 ± 13.5</td>
<td>48 ± 12.7</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>125 (47)</td>
<td>48 (49)</td>
</tr>
<tr>
<td>African American</td>
<td>54 (20)</td>
<td>17 (18)</td>
</tr>
<tr>
<td>Latino</td>
<td>77 (29)</td>
<td>27 (29)</td>
</tr>
<tr>
<td>Diffuse cutaneous involvement</td>
<td>156 (59)</td>
<td>–</td>
</tr>
<tr>
<td>Disease duration, mean ± SD years†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definition 1</td>
<td>2.5 ± 1.6</td>
<td>–</td>
</tr>
<tr>
<td>Definition 2</td>
<td>4.5 ± 5.4</td>
<td>–</td>
</tr>
<tr>
<td>Autoantibody positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAs</td>
<td>32 (12)</td>
<td>–</td>
</tr>
<tr>
<td>Antitopoisomerase</td>
<td>49 (18)</td>
<td>–</td>
</tr>
<tr>
<td>Anti–RNAP III</td>
<td>61 (23)</td>
<td>–</td>
</tr>
<tr>
<td>Anti–U1 RNP</td>
<td>30 (11)</td>
<td>–</td>
</tr>
<tr>
<td>Treatment with immunosuppressive agents</td>
<td>82 (32)</td>
<td>–</td>
</tr>
</tbody>
</table>

* Except where indicated otherwise, values are the number (%) of subjects. ACAs = anticentromere antibodies; anti–RNAP III = anti–RNA polymerase III.
† For disease duration, definition 1 was calculated as number of years from the onset of the first non–Raynaud’s phenomenon symptom, while definition 2 was calculated as number of years from the onset of the first symptom (Raynaud’s phenomenon or non–Raynaud’s phenomenon) attributable to SSc.
did not yield significant results. Therefore, the patients were not subgrouped according to treatment status, and this variable was included as a potential confounder in the multivariable models.

We also performed Bonferroni corrections for multiple comparisons, based on 3 independent comparisons (MCP-1, ITAC, and IP-10) in the gene expression/chemokine analysis, and 2 independent comparisons (ITAC and IP-10) for the SSc subtype/disease severity analysis.

Subsequently, linear regression was used to examine the association of the baseline IFN-inducible chemokine levels with the percentage change in MRSS and FVC between the baseline and followup visit at year 1, calculated as \( \frac{(\text{level}_{\text{baseline}} - \text{level}_{\text{year 1}})}{\text{level}_{\text{baseline}}} \). This was examined to investigate the predictive significance of the chemokine composite score in predicting any short-term change in skin involvement and severity of ILD in patients with SSc.

Longitudinal comparisons of the log-transformed IFN-inducible chemokine levels were performed by paired \( t \)-tests. Furthermore, the percentage change in chemokine levels, calculated as \( \frac{(\text{level}_{\text{baseline}} - \text{level}_{\text{followup}})}{\text{level}_{\text{baseline}}} \), was assessed for correlations with the percentage change in FVC and MRSS, by linear regression and Pearson’s correlation analysis. Two-sided \( P \) values less than 0.05 were considered significant. All analyses were performed using the Stata/SE statistical program (release 11.2; StataCorp).

RESULTS

Correlation of plasma IFN-inducible chemokine levels with the IFN gene signature. We examined 24 patients with SSc by global gene expression profiling, qPCR, and multiplex chemokine assays, using concomitantly obtained samples of whole blood RNA and plasma (a description of the demographic and clinical characteristics of these patients is available at http://www.uth.tmc.edu/scleroderma). None of these 24 patients were being treated with immunosuppressive agents at the time of blood withdrawal. The 24 patients were a subgroup of the SSc cohort in our large, whole blood gene expression study (6), and each of these patients had a concomitantly collected plasma sample available for analysis.

The results of analyses assessing the correlations of plasma levels of the investigated chemokines with the microarray and qPCR IFN gene expression scores are shown in Table 1. Plasma levels of IP-10 and I-TAC correlated significantly with both the microarray IFN score (\( P = 0.007, \rho = 0.536 \) and \( P = 0.01, \rho = 0.518 \), respectively) and the qPCR IFN score (\( P = 0.003, \rho = 0.581 \) and \( P = 0.009, \rho = 0.521 \), respectively). However, no significant correlation was detected between the MCP-1 plasma level and either the microarray IFN score (\( P = 0.27, \rho = 0.234 \)) or the qPCR IFN score (\( P = 0.126, \rho = 0.321 \)). The composite IFN-inducible chemokine

![Figure 1](http://www.uth.tmc.edu/scleroderma)
score for IP-10 and I-TAC showed a stronger correlation with the microarray IFN score ($\rho = 0.612$) and the qPCR IFN score ($\rho = 0.620$) than did the individual IP-10 and I-TAC chemokine levels. Addition of MCP-1 to the composite chemokine score weakened the correlation. Therefore, only the composite IFN-inducible chemokine score for IP-10 and I-TAC plasma levels was utilized in subsequent analyses. Of note, exclusion of the 4 patients with late SSc (i.e., disease duration longer than 5 years from the first non–Raynaud’s phenomenon symptom; these samples were obtained on followup visits in the GENISOS cohort) did not change the above-described correlations (results available at http://www.uth.tmc.edu/scleroderma).

**Comparison of IFN-inducible chemokine levels between SSc patients and unaffected controls.** The IFN-inducible chemokine scores were determined in all plasma samples obtained at baseline from SSc patients enrolled in the GENISOS cohort ($n = 266$) and from 97 matched controls. The demographic and clinical characteristics of these patients and control subjects at the time of blood withdrawal are shown in Table 2. Patients with SSc had higher circulating levels of IP-10 ($P < 0.001$) and I-TAC ($P < 0.001$) than their age-, sex-, and ethnicity-matched unaffected controls (Figures 1A and B). The IFN-inducible chemokine score was also higher in patients with SSc compared to controls ($P < 0.001$) (Figure 1C). In this analysis, 39.2% of patients had an IFN-inducible chemokine score that was considered positive when the composite score was dichotomized based on the 95th percentile level in unaffected controls.

**Association of disease subtypes with plasma IFN-inducible chemokines.** Table 3 shows the univariable and multivariable associations of disease subtypes with IFN-inducible chemokine levels in the 266 SSc patients enrolled in the GENISOS cohort. After adjustment for potential confounders (age at enrollment, sex, ethnicity, and treatment with immunosuppressive agents) in the multivariable model, we found that neither the disease type (limited versus diffuse) nor disease duration correlated with the IP-10 level, I-TAC level, or IFN-inducible chemokine score.

The presence of anti–U1 RNP antibodies correlated with higher levels of IP-10, I-TAC, and the IFN-inducible chemokine score ($P < 0.001, P = 0.018$, and $P < 0.001$, respectively). In contrast, presence of anti–RNAP III antibodies correlated with lower plasma levels of I-TAC and a lower IFN-inducible chemokine score ($P = 0.004$ and $P = 0.003$, respectively). None of the other SSc-related autoantibodies showed a correlation with the investigated chemokines. The negative correlation of anti–RNAP III antibodies with the IFN-inducible chemokine score remained significant ($P = 0.05$) even after patients with anti–U1 RNP antibodies were excluded from the analysis. The inclusion of patients with 2 SSc-related autoantibodies ($n = 7$) or those with anti-Ro antibodies ($n = 10$) did not change the above-observed significant associations with anti–U1 RNP and anti–RNAP III (results not shown).

Table 3. Association of disease subtypes with plasma levels of interferon (IFN)–inducible chemokines and the IFN-inducible chemokine score*  

<table>
<thead>
<tr>
<th>Disease subtype</th>
<th>IP-10</th>
<th>I-TAC</th>
<th>IFN-inducible chemokine score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_{uni}$</td>
<td>$P_{multi}$</td>
<td>Mean difference (95% CI)</td>
</tr>
<tr>
<td>Disease duration†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definition 1</td>
<td>0.126</td>
<td>0.204</td>
<td>$-0.05$ ($-0.14, 0.03$)</td>
</tr>
<tr>
<td>Definition 2</td>
<td>0.470</td>
<td>0.482</td>
<td>$-0.01$ ($-0.03, 0.02$)</td>
</tr>
<tr>
<td>Disease type‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoantibody positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACAs</td>
<td>0.219</td>
<td>0.318</td>
<td>$-0.21$ ($-0.62, 0.20$)</td>
</tr>
<tr>
<td>Antitopoisomerase</td>
<td>0.548</td>
<td>0.258</td>
<td>$-0.21$ ($-0.57, 0.15$)</td>
</tr>
<tr>
<td>Anti–RNAP III</td>
<td>0.219</td>
<td>0.096</td>
<td>$-0.27$ ($-0.58, 0.05$)</td>
</tr>
<tr>
<td>Anti–U1 RNP</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>$0.25$ ($0.38, 0.12$)</td>
</tr>
<tr>
<td>Fibrillarin</td>
<td>0.776</td>
<td>0.635</td>
<td>$0.11$ ($-0.38, 0.57$)</td>
</tr>
</tbody>
</table>

* IP-10 = IFN-γ-inducible protein 10; $P_{uni}$ = $P$ value from univariable model; $P_{multi}$ = $P$ value from multivariable model with adjustment for age at enrollment, sex, ethnicity, and treatment with immunosuppressive agents; 95% CI = 95% confidence interval; I-TAC = IFN-inducible T cell α chemotactrant; $P_{multiscor}$ = $P$ value from multivariable model with correction for multiple comparisons; ACAs = anticientromere antibodies; anti–RNAP III = anti–RNA polymerase III.

† For disease duration, definition 1 was calculated as number of years from the onset of the first non–Raynaud’s phenomenon symptom, while definition 2 was calculated as number of years from the onset of the first symptom (Raynaud’s phenomenon or non–Raynaud’s phenomenon) attributable to systemic sclerosis.

‡ Limited versus diffuse.
Correlation of IFN-inducible chemokines with disease severity. Table 4 shows the univariable and multivariable associations of plasma levels of IFN-inducible chemokines with disease severity in the 266 SSc patients. IP-10 levels were correlated with a higher FVC (P = 0.003), lower DLCO (P = 0.002), and higher creatine kinase level (P < 0.001). The IFN-inducible chemokine score correlated with a lower FVC (P = 0.013) and lower DLCO (P = 0.008) and higher creatine kinase level (P = 0.002).

As shown in Table 4, the association with disease severity scores for components of the Medsger Severity Index paralleled the above-mentioned findings. Specifically, the IFN-inducible chemokine score was associated with severity scores for muscle involvement (P = 0.006) and lung involvement (P = 0.021). Furthermore, the composite chemokine score showed a trend toward association with the skin component of the Medsger Severity Index (P = 0.073). Adjustment for age at enrollment, sex, ethnicity, disease duration, and treatment with immunosuppressive agents, as well as correction for multiple comparisons, did not change the above-observed associations (Table 4).

After exclusion of patients with anti-U1 RNP antibodies, all of the above-mentioned correlations remained significant (results not shown) and the correlation between the IFN-inducible chemokine score and higher MRSS also became significant (r = 0.16, P = 0.018). Furthermore, a subgroup analysis based on the disease type (limited or diffuse SSc) did not show any association of the chemokine score with the MRSS that was more significant than that in the overall cohort.

We further investigated whether the IFN-inducible chemokine score is associated with the presence of ILD. A combined outcome for ILD, indicated by increased reticular markings on chest radiograph, rales on physical examination, or FVC of <70% predicted (all of which are known predictors of the presence of ILD-related changes on high-resolution chest computed tomography [17]), was examined. A higher composite IFN-inducible chemokine score was associated with this ILD combined outcome (P = 0.048). Furthermore, a higher composite chemokine score was associated with severe restrictive lung disease, defined as an FVC of <50% predicted (P = 0.045).

The IFN-inducible chemokine levels in the SSc plasma samples at baseline were associated with a lower lymphocyte count (r = −0.19, P = 0.004), but not with the total white cell count (P = 0.59), the hematocrit level (P = 0.586), or the platelet count (P = 0.313). The negative association with the lymphocyte count was independent of age at enrollment, sex, ethnicity, disease duration, and treatment with immunosuppressive agents (P = 0.008 in the multivariable model). Neither the combined baseline IFN-inducible chemokine levels nor the IP-10 and I-TAC levels assessed individually correlated significantly with the short-term change in FVC or MRSS.

Progression of IFN-inducible chemokine score over time, and clinical correlations with the change in score over time. A follow-up plasma sample was available from 63 patients with SSc. Among this subgroup, the mean ± SD time-in-study was 3.1 ± 1.2 years. Neither the IP-10 plasma levels nor the I-TAC plasma levels changed significantly over time (P = 0.977 and P =
an association between anti–RNAP III antibodies and previous gene expression study (6), we did not observe a significant correlation with the change in MRSS, creatine kinase level, or FVC (results not shown).

Similarly, the IFN-inducible chemokine score did not change significantly over time ($P = 0.621$). As expected, the baseline IFN-inducible chemokine score correlated significantly with the score at followup ($r = 0.39, P = 0.002$). We found no correlation between change in the IFN-inducible chemokine score and change in the FVC or creatine kinase level over time ($r = -0.08, P = 0.598$ and $r = -0.06, P = 0.719$, respectively), whereas there was a trend toward a correlation between change in the IFN-inducible chemokine score and change in the MRSS ($r = 0.23, P = 0.084$).

Finally, we excluded all patients who were taking immunosuppressive agents at the baseline visit and/or followup visit, in order to remove the confounding effect of treatment. In the remaining 35 patients, the IFN-inducible chemokine score did not change significantly over time ($P = 0.662$), and the followup score correlated significantly with the baseline score ($r = 0.48, P = 0.003$). Moreover, change in the IFN-inducible chemokine score did not correlate with change in the FVC ($r = -0.16, P = 0.348$), change in the creatine kinase level ($r = -0.31, P = 0.119$), or change in the MRSS ($r = 0.16, P = 0.406$).

DISCUSSION

We developed a composite plasma IFN-inducible chemokine score that correlated with the IFN gene expression signature and the disease severity in SSc. To our knowledge, this is the first published study to show a correlation of the IFN-inducible chemokine plasma levels with the IFN gene expression signature in SSc. Furthermore, availability of plasma samples in the GENISOS cohort enabled us to examine the correlation of the IFN-inducible chemokine score with subtypes and severity of SSc in a large and well-characterized patient population with early disease. We were able to demonstrate, for the first time, an association between the IFN-inducible chemokine score and the severity of lung and muscle involvement, and confirmed previous observations that linked IFN activity to severity of skin involvement in SSc (3,18).

We observed a negative association of the chemokine score with anti–RNAP III antibodies, independent of other potential confounding factors. In our previous gene expression study (6), we did not observe an association between anti–RNAP III antibodies and the IFN gene signature, because that study was underpowered, as only 15 patients with these antibodies were investigated. The other gene expression studies have not investigated an association of the IFN signature with anti–RNAP III antibodies (1,4,5,19). A negative association of anti–RNAP III antibodies with the IFN chemokine score indicates that dysregulation of IFN pathways is less likely to play a pathogenic role in this autoantibody subgroup of patients with SSc.

Of interest, anti–RNAP III antibodies are highly associated with extensive skin involvement, but severe ILD is infrequent in this subgroup. In agreement with published data (20), 82% of patients with anti–RNAP III antibodies had diffuse cutaneous involvement, but only 2% of them had severe restrictive lung disease in the GENISOS cohort. Further studies are needed to examine whether lack of IFN activation contributes to the observed dissociation between fibrosis in the skin and pulmonary tissue in this subgroup of patients with SSc. Anti–RNAP III antibodies are also associated with scleroderma renal crisis. Of note, we did not observe an association of IFN-inducible chemokine scores with severity of renal involvement in our cohort.

In addition, we confirmed previous observations that anti–U1 RNP antibodies are associated with increased IFN activity in patients with SSc (6,19). Finally, confirming the findings of previous studies, we did not find an association of the IFN-inducible chemokine score with disease type (limited versus diffuse) (1,4–6) or duration of disease (1,4,6).

The easier accessibility of plasma samples compared to peripheral blood RNA samples enabled us to assess correlations of the IFN-inducible chemokine plasma levels with clinical features in a large, well-characterized cohort of patients with early SSc. The IFN-inducible chemokine score correlated with concomitant severity of skin, lung, and muscle involvement. This correlation was independent of potential demographic confounders, treatment with immunosuppressive agents, and disease duration. Notably, the observed association with markers of clinical severity remained significant even after exclusion of patients with anti–U1 RNP antibodies. This indicates that the association of IFN-inducible chemokines with more severe forms of SSc is not mainly driven by the features of mixed connective tissue disease in some patients.

Our findings also confirmed those from previous studies indicating that transcript levels of IFN-inducible genes are correlated with the severity of skin disease in patients with SSc (3,18). However, this is the first study to show an association between the IFN-inducible
chemokine score and the severity of lung and muscle disease in SSc. This association with severity of lung involvement is especially important because this disease manifestation is the primary cause of SSc-related mortality (21,22). However, the baseline composite chemokine score was not predictive of any short-term change in the FVC in the present study, although future longitudinal studies with combined analysis of longitudinal measurements (serially obtained FVC measurements) and survival data are needed to investigate the predictive significance of the composite score as a predictor for long-term progression of SSc-related ILD.

Our findings provide further support for the deleterious effects of IFN in SSc. The development of SSc has been reported in patients undergoing IFNα treatment (23,24). Furthermore, a randomized, placebo-controlled trial of subcutaneous IFNs in patients with early SSc showed that treatment with IFNα resulted in worsening lung function and a trend toward skin deterioration (25). The potential role of IFN in the pathogenesis of SSc has led to an ongoing phase I study of an anti-IFNα monoclonal antibody, sifalimumab, for treatment of this disease. The IFN-inducible chemokine score developed in this study might be helpful in identifying SSc patients who would benefit from this treatment modality.

Our IFN-inducible chemokine score comprised both IP-10 and I-TAC, both of which are ligands for CXCR3. An elevation in I-TAC levels in the plasma of patients with SSc is a novel observation. In agreement with our findings, higher levels of IP-10 have been observed previously in patients with SSc compared to unaffected controls (19,26,27). Higher IP-10 levels were also associated with the presence of ILD, defined as presence of ground glass and/or interstitial fibrosis detected by high-resolution chest computed tomography. However, the correlation of IP-10 levels with the severity of ILD (measured with the FVC) has not been investigated previously.

Type I and type II IFNs can stimulate overlapping series of genes, including IP-10 and I-TAC (28). Therefore, we cannot discern whether the observed association of IFN-inducible chemokine plasma levels with the disease severity is driven by either type I IFN or type II IFN. Furthermore, it is likely that these chemokines are not exclusively induced by IFN, because the redundancies in biologic pathways enable induction of key chemokines by several upstream molecules. For example, Weckerle et al recently demonstrated a moderate cross-sectional correlation between tumor necrosis factor α levels and IFNα serum activity in patients with SLE (29).

In agreement with our findings, previous studies have shown that IP-10 and I-TAC levels correlated with the IFN gene signature in patients with SLE (7) and those with dermatomyositis (9). However, contrary to those studies (7,9), we did not observe a correlation between the MCP-1 levels and the IFN gene signature. It is possible that MCP-1 levels in patients with SSc are mainly regulated by molecules involved in other pathways. In fact, MCP-1 was recently identified as a key IL-13–regulated cytokine in a murine model of fibrosis and in skin biopsy samples from patients with SSc (30).

The longitudinal examination of IFN-inducible chemokine plasma levels indicated that they did not significantly change over time, and that the chemokine levels in baseline and followup samples from SSc patients correlated with each other. Furthermore, the changes in chemokine levels did not correlate with progression of the FVC (which would indicate increased severity of ILD). This suggests that the IFN-inducible chemokine score is a marker for a more severe subtype of SSc, but is not a dynamic measure of disease activity in SSc-related ILD. This notion was also supported by the fact that the chemokine score, when assessed at the cross-sectional level, did not correlate with disease duration. However, we did observe a trend toward a correlation between change in the IFN-inducible chemokine score and change in the MRSS. This weak correlation was not confirmed in the subgroup of patients not treated with immunosuppressive agents. Based on our present findings, we cannot exclude the possibility that there is a weak correlation between change in the IFN-inducible chemokine score and skin involvement. Larger, longitudinal studies are needed to investigate this possibility.

Of note, fibrotic processes in SSc are often irreversible, and thus it is more difficult to identify dynamic markers of disease activity in SSc when compared to other autoimmune diseases, such as SLE or dermatomyositis. The investigated disease severity markers, such as the FVC and MRSS, reflect disease damage (irreversible) as well as disease activity (reversible). Nevertheless, we believe stable markers of disease severity are important for identifying patients who may benefit from more aggressive monitoring and treatment.

Our study was conducted in a multiethnic cohort, which could increase the generalizability of our findings across investigated ethnic groups. Furthermore, the enrollment of only patients with early disease decreased the likelihood that our results were influenced by sur-
vival bias. The careful prospective collection of demographic and medical data, including the medication regimen, also enabled us to adjust for several potential confounding variables.

The current study has some limitations. The GENISOS cohort is derived from 3 tertiary care centers in Texas, which partially explains the high proportion of patients with diffuse cutaneous disease in this study. Although we were able to link the IFN-inducible chemokine plasma levels to disease severity in our patients with SSC, their actual contribution to various disease manifestations of SSC needs to be explored in future studies of pathogenic mechanisms.

Furthermore, in our longitudinal study, only a subgroup of patients who had both a baseline and followup plasma sample available could be studied. In addition, the lack of a validated disease activity scale in SSC has hampered identification of biomarkers that track dynamic changes in disease activity. For example, the FVC and MRSS are measures of both disease activity and disease damage, because fibrotic changes in the lung and skin are partially irreversible.

In summary, we found that the composite chemokine score of IP-10 and I-TAC plasma levels showed a correlation with the IFN gene expression signature in patients with SSC. This IFN-inducible chemokine score correlated with the severity of lung, skin, and muscle involvement, even after adjustment for potential demographic and clinical confounders. Furthermore, the IFN-inducible chemokine score did not change significantly over time, suggesting that this composite chemokine score can serve as a stable marker for a more severe subtype of SSC. This finding may lead to more effective and focused monitoring and treatment of patients with SSC.

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.

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Analysis and interpretation of data. Liu, Mayes, Tan, Wu, Reveille, Draeger, Assassi.

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