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# Serum CCL24 as a Biomarker of Fibrotic and Vascular Disease Severity in Systemic Sclerosis

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**Background.** Systemic sclerosis (SSc) is a heterogeneous disease, characterized by variable tissue and vascular fibrosis in the context of autoimmune activation. CCL24 (or Eotaxin2) has been shown to promote microangiopathic, proinflammatory, and profibrotic processes in preclinical models of SSc. Here, we study serum CCL24 levels in a real-life cohort of patients with SSc, to determine its distribution across disease features and its value in predicting disease progression and related mortality.

**Methods.** Serum CCL24 was assessed in an observational cohort of consecutively enrolled patients with SSc. A high CCL24 cutoff was defined based on its distribution in a matched cohort of healthy controls. Disease progression and mortality were analyzed from the date of serum assessment.

**Results.** Two-hundred thirteen consecutively enrolled patients with SSc were included in this analysis. Median disease duration was six years (interquartile range 3–14), 28.6% of patients presented with interstitial lung disease (ILD), 46.9% had digital ulcers, and 25.3% showed high CCL24 serum concentration. High-CCL24 patients were more frequently male and positive for anti–scl-70, with a diagnosis of ILD and synovitis ($P < 0.05$ for all). Notably, high-CCL24 patients had lower diffusion of carbon monoxide and higher prevalence of digital ulcers, telangiectasias, and calcinosis ($P < 0.05$ for all). In a longitudinal setting, high CCL24 was associated with greater lung function decline and with higher disease-related mortality.

**Conclusion.** Serum CCL24 is a biomarker of disease severity across fibrotic and vascular disease manifestations. These data support the development of therapies targeting CCL24 as a novel comprehensive therapeutic target in SSc.

## INTRODUCTION

Systemic sclerosis (SSc) is a chronic rheumatic disease characterized by poor survival rate due to its potential involvement in nearly every organ system, including the skin. Despite the clinical heterogeneity observed in patients with SSc, they share a variable degree of common pathophysiologic changes, such as endothelial damage and dysfunction, abnormal immune activation, and excessive fibrosis in affected organs.[1]

Cardiopulmonary and vascular complications are the leading cause of mortality among patients with SSc.[2] The disease progression and outcomes can vary significantly even among patients with similar clinical profiles. Therefore, an accurate prognostic evaluation is valuable in identifying patients at a higher risk of deterioration and those who would benefit most from timely or innovative therapeutic interventions.

CCL24 (or Eotaxin2) has been identified as a novel target that plays a dual role in promoting proinflammatory and profibrotic processes in SSc.[3,4] Previous studies have shown elevated levels of CCL24 in the serum of patients with SSc, as well as in the perivascular inflammatory infiltrate of the skin in cases of diffuse cutaneous SSc. The CCL24 receptor, CCR3, is also overexpressed in fibroblasts and endothelial cells within the same cutaneous biopsies. Moreover, blocking CCL24 prevents and mitigates skin and pulmonary fibrosis in murine models, also leading to a reduction in cellularity in the bronchoalveolar lavage fluid (BALF).[5]

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**SIGNIFICANCE & INNOVATIONS**

- One in four patients with systemic sclerosis (SSc) has a high CCL24 serum concentration in a real-life population.
- High CCL24 serum levels are associated with male sex, anti–SCL-70 positivity, interstitial lung disease (ILD) calcinosis, telangiectasia, and a history of digital ulcers and synovitis.
- In patients with SSc and ILD, a high CCL24 status is linked with a more significant decline in lung function over two years.
- High CCL24 status correlates with an increased 10-year SSc-related mortality rate.

The primary objective of this study is to investigate the relationship between serum CCL24 levels and disease characteristics in a comprehensive real-life cohort of patients with SSc. Furthermore, the study aims to determine whether baseline serum CCL24 levels can predict clinical progression of interstitial lung disease (ILD) in patients with SSc and to evaluate its association with disease-related mortality.

## METHODS

**Study design and patient cohort.** This study was conducted as a retrospective analysis of an observational cohort consented for the study of factors contributing to disease progression (English Health Research Authority RR10/9608 and 15/NE/0211) with prospectively collected clinical data. The research encompassed both a baseline cross-sectional description and a longitudinal assessment, serving as a predefined analysis of an observational cohort. The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) and the Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) initiative guidelines were used to define the study design and report results.[6,7]

For inclusion in the analysis, consecutive patients enrolled in the observational study between January 2012 and December 2018 were included if they fulfilled American College of Rheumatology/EULAR classification criteria for SSc.[8] The study included a control group of healthy patients (health controls [HCs]) derived from propensity score matching at a 1:5 ratio based on age and sex. The outcome of the process was assessed using the standardized mean difference, with values lower than 0.1 being used as the recommended threshold to declare balance.[9] The control group was used to define a normal reference range of CCL24.

**Clinical assessment and outcome measures.** The clinical annotation in the cohort followed the European Scleroderma Trials and Research group Minimal Essential Dataset guidelines,[10] including the collection of medical history, physical examinations, and data from specific diagnostic tests within 12 weeks from blood collection for CCL24 assessment. The collection of clinical variables was standardized to prevent information biases and missing data. Specifically, the severity of skin sclerosis was measured using the modified Rodnan skin score (mRSS), whereas cardiopulmonary involvement was evaluated through annual pulmonary function tests (PFTs) and transthoracic echocardiograms. High-resolution computed tomography (HRCT), right-heart catheterization (RHC), and cardiac magnetic resonance imaging (CMRI) were performed either at baseline or during follow-up as clinically indicated and recorded in the database.

For descriptive purposes in this study, medication exposure at baseline was categorized into vasoactive treatments (calcium channel blockers, endothelin receptor antagonists, phosphodiesterase-5 inhibitors, and iloprost) and immunosuppressive treatments (cyclophosphamide, mycophenolate mofetil, azathioprine, methotrexate, rituximab, and tocilizumab).

Follow-up assessments were recorded in the database according to routine clinical follow-up schedule with a minimum of six monthly intervals. The outcome measures chosen for the study included forced vital capacity (FVC) relative change at 12 months, occurrence of progressing fibrotic ILD (PF-ILD) by month 24, based on the combination of PFTs deterioration, HRCT changes, and respiratory symptoms worsening according to the Erice research working group on ILD definition.[11] Moreover, any deterioration in FVC, along with the establishment of a 5% and 10% relative reduction, within 12 months were considered.[12] In the subgroup of patients classified with diffuse cutaneous variant according to LeRoy's classification, worsening of the mRSS at 12 months was defined as a minimal clinically important difference (MCID). This was specified as a 5-point increase for patients with baseline mRSS values <20 and a 25% difference within a 12-month period for patients with baseline mRSS ≥20, as previously described.[13]

Finally, the analysis included the 10-year SSc-related mortality. The cause of death was determined based on the available digital clinical files for each enrolled patient. Each death was classified as either an SSc-related death or a death related to an alternative cause. SSc-related deaths included scleroderma renal crisis, pneumonia in patients with severe ILD or pulmonary arterial hypertension (PAH), right-heart failure, end-stage respiratory failure, acute ischemic event, or major arrhythmia in patients with severe ILD, RHC-proven PAH, or CMRI-defined primary SSc heart involvement at the last available evaluation. Severe SSc-associated ILD was defined as FVC ≤80% and hemoglobin-adjusted alveolar diffusion of carbon monoxide ($DL_{CO}$) ≤50% at the most recent evaluation.[14] Deaths due to cancer, infective complications, or cardiovascular events in patients without severe cardiopulmonary involvement, as well as deaths classified as senectus (death at home in patients over 80 without any of the previous conditions), were not considered SSc-related.

**CCL24 assessment.** Serum samples were collected at baseline and processed according to a standardized protocol, subsequently aliquoted, stored in –80°C freezers, and shipped on dry ice. CCL24 serum concentration were performed by the CLIA certified Myriad Rules-Based Medicine using Luminex Multi-Analyte Profiling multiplexed immune assay.

**Statistical analysis.** Categorical variables were presented as numbers and percentages, and continuous variables were reported as mean ± SD or median with interquartile range (IQR) based on the normality of the data, which was assessed through inspection of quantile-quantile plots. The threshold to distinguish between high serum CCL24 levels and low serum CCL24 levels was one SD above the mean of serum natural logarithm-transformed CCL24 levels in the matched HC group.

Comparisons between patient groups were conducted using the chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney U test or *t*-test, depending on the data distribution and variance homogeneity assessed by Levene's test, for continuous variables. The relationship between CCL24 and FVC changes was explored using Spearman R. A binomial logistic regression was performed to ascertain the association of CCL24 at baseline on the likelihood that participants experience PF-ILD according to Erice criteria or mRSS worsening MCID on follow-up. Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

The Kaplan-Meier method with log-rank testing was conducted to determine if there were differences in the overall 10-year survival distributions according to baseline CCL24 status. A 10-year competing risk survival analysis was performed[15] to investigate SSc-related death from alternative causes of death. The cumulative incidence function was applied to account for the competing event, with deaths due to other causes considered as competing risks for SSc-related mortality. The cumulative incidence of the primary endpoint was quantitatively compared between groups using Fine-Gray competing risk regression with subhazard ratio (sHR) and 95% CIs as summary statistics.

Models adjusted for demographic variables (age, sex) and major clinical prognostic factors (LeRoy, disease duration up to three years) were proposed for logistic regression and survival analysis. Statistical significance was defined as $P < 0.05$ for all analyses, and all tests were two-tailed. Data analysis was performed using RStudio (version 2023.03.0).

**Sample size.** A minimum sample size of 187 patients with SSc and a minimum sample 37 HCs was determined, assuming a minimal effect size of 0.65 based on previously published comparisons of serum CCL24 between patients with SSc and controls.[5] A pragmatic allocation ratio of 5:1 was applied between the two groups. The study aimed to achieve a power of 0.9 with a significance level (α) set at 0.05. The sample size was increased by approximately 15% to account for possible loss to follow-up. The sample size calculations were performed with G*Power 3.1.9.6.

**Patient involvement.** Although patients were not directly involved in the design of the study, the study is part of a need prioritized by patient in the STRIKE observational cohort, to identify blood test predictive of disease activity and progression. All patients were offered to be provided with the results of the study upon their request, and each result is disseminated in yearly public and patient engagement events.

The study was conducted within the ethical approval from the English Health Research Authority STRIKE: Stratification for risk of progression in SSc (15/NE/0211) and RR10/9608. The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

## RESULTS

**Comparison of serum CCL24 levels between patients affected by SSc and HCs.** A total of 224 patients fulfilling SSc classification criteria were consecutively enrolled between January 2012 and December 2018. Blood samples were unavailable for 11 patients; hence CCL24 assessment and final analysis was performed on 213 patients. Follow-up information was adequate to verify the outcome measures for all patients. The clinical characteristics of the patients with SSc included in the final analysis are reported in Table 1.

Forty-three individuals were enrolled as HCs. The demographic characteristics of these HCs and patients with SSc are detailed in Supplementary Table 1. Median (IQR) CCL24 serum concentration in HCs was 885 (570–998) pg/mL, with a nonnormal distribution. According to the distribution of the natural logarithm of CCL24, its serum concentration was considered "high" when values exceeded 1,288 pg/mL, the cutoff corresponding to one SD above the mean value (Supplementary Figure 1). The range of values among patients with SSc was broader compared to HCs, with 54 of the 213 patients (25.4%) exhibiting a high CCL24 status (Figure 1A).

**Clinical characteristics and serum CCL24 levels in patients with SSc.** Median (IQR) serum CCL24 levels were higher in male patients (1,164 [706–1,690] vs 707 [487–1,180] pg/mL), in those with a history of synovitis (1,224 [735–1,803] vs 739 [487–1,120] pg/mL), and in those with ILD as demonstrated by HRCT (964 [629–1,510] vs 686 [485–1,075] pg/mL) ($P < 0.01$ for all). Additionally, patients who tested positive for anti–Scl-70 also exhibited higher serum CCL24 levels compared to those testing negative (1,047 [IQR 607–1,585] vs 739 [IQR 492–1,175], $P < 0.05$). There were not significant differences in serum CCL24 across cutaneous subsets (817 [IQR 481–1,550] vs 737 [IQR 497–1,160] pg/mL) (Figure 1B).

**Table 1.** General characteristics of patients with SSc and comparison between baseline Hi and Lo CCL24 status groups*

| Clinical variables | Overall | Baseline CCL24 status | | *P* value |
|---|---|---|---|---|
| | | Hi CCL24 | Lo CCL24 | |
| | N = 213 | N = 54 | N = 159 | |
| Age, mean ± SD, y | 54.2 ± 12.4 | 55.2 ± 12.0 | 53.9 ± 12.6 | 0.5 |
| BMI, kg/m$^2$, mean ± SD | 26.4 ± 5.3 | 25.6 ± 5.7 | 26.7 ± 5.1 | 0.2 |
| Male sex, n (%) | 28 (13.1) | 11 (20.4) | 17 (10.7) | 0.069 |
| LeRoy diffuse cutaneous variant, n (%) | 51 (23.9) | 18 (33.3) | 33 (20.8) | 0.061 |
| Disease duration, median (IQR), y | 6.0 (3.0–14.0) | 5.0 (2.0–13.0) | 7.0 (3.0–14.0) | 0.2 |
| Disease duration ≤3 y, n (%) | 73 (34.3) | 22 (40.7) | 51 (32.1) | 0.2 |
| Anticentromere positive, n (%) | 110 (51.6) | 24 (44.4) | 86 (54.1) | 0.2 |
| Anti–Scl-70 positive, n (%) | 38 (17.8) | 14 (25.9) | 24 (15.1) | 0.072 |
| Active-late capillaroscopy pattern,[a] n (%) | 110 (67.9) | 29 (74.4) | 81 (65.9) | 0.3 |
| Late capillaroscopy pattern,[a] n (%) | 54 (33.1) | 17 (42.5) | 37 (30.1) | 0.15 |
| mRSS, median (IQR) | 2.0 (0.0–4.0) | 3.0 (2.0–4.8) | 2.0 (0.0–4.0) | 0.048 |
| ILD on HRCT, n (%) | 61 (28.6) | 22 (40.7) | 39 (24.5) | 0.023 |
| PAH on RHC, n (%) | 18 (8.5) | 6 (11.1) | 12 (7.5) | 0.4 |
| Baseline FVC, mean ± SD, % of predicted | 103.9 ± 22.0 | 101.7 ± 21.7 | 104.6 ± 22.1 | 0.4 |
| Baseline TLC, mean ± SD % of predicted | 96.7 ± 16.5 | 97.4 ± 18.1 | 96.5 ± 16.0 | 0.7 |
| Baseline DLco, mean ± SD, % of predicted | 64.7 ± 15.5 | 60.2 ± 14.5 | 66.2 ± 15.6 | 0.015 |
| Baseline FVC ≤80% of predicted, n (%) | 38 (17.9) | 11 (20.4) | 27 (17.1) | 0.6 |
| Baseline DLco ≤50% of predicted, n (%) | 37 (17.6) | 14 (26.4) | 23 (14.6) | 0.052 |
| Baseline FVC ≤80% or DLco ≤50% of predicted, n (%) | 60 (98.4) | 22 (100.0) | 38 (97.4) | >0.9 |
| Digital ulcers (ever), n (%) | 100 (46.9) | 32 (59.3) | 68 (42.8) | 0.036 |
| Digital ulcers (current), n (%) | 30 (14.1) | 10 (18.5) | 20 (12.6) | 0.3 |
| Skin calcinosis, n (%) | 117 (54.9) | 36 (66.7) | 81 (50.9) | 0.045 |
| Skin telangiectasia, n (%) | 137 (64.3) | 41 (75.9) | 96 (60.4) | 0.039 |
| Synovitis (ever), n (%) | 16 (7.5) | 8 (14.8) | 8 (5.0) | 0.032 |
| Myositis (ever), n (%) | 18 (8.5) | 5 (9.3) | 13 (8.2) | 0.8 |
| Immunosuppressive treatment, n (%) | 59 (27.7) | 23 (42.6) | 36 (22.6) | 0.005 |
| Vasoactive treatment, n (%) | 141 (66.2) | 38 (70.4) | 103 (64.8) | 0.5 |
| CCL24, median (IQR), pg/mL | 748 (496–1,290) | 1,710 (1,505–2,260) | 559 (445–811) | <0.001 |
| Ln CCL24, mean ± SD | 6.7 ± 0.7 | 7.6 ± 0.3 | 6.4 ± 0.4 | <0.001 |

* High CCL24 status was defined as serum values exceeding 1,288 pg/mL. BMI, body mass index; DLco, diffusing capacity of the lung for carbon monoxide; Hi, high; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; FVC, forced vital capacity; IQR, interquartile range; Lo, low; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; RHC, right heart catheterization; TLC, total lung capacity.
[a] Capillaroscopy status was missing for 12 patients, with 6 from each group.

The comparison between patients with high and low CCL24 status is presented in Table 1. A high CCL24 status was consistently associated with a higher prevalence of ILD and synovitis, more severe skin fibrosis according to the mRSS, and worse impairment of lung microcirculation as measured by DLco. Furthermore, a high CCL24 status was associated with a digital ulcer disease (history or current digital ulcers), skin telangiectasias, and calcinosis ($P < 0.05$ for each comparison). Lastly, patients with a high CCL24 status were more commonly treated with immunosuppressants ($P < 0.01$).

**Baseline serum CCL24, ILD functional deterioration, and progression of skin diffuse cutaneous subset.** Sixty-one patients (28.6%) were diagnosed with ILD, confirmed by HRCT; 12-month PFTs were available for 53 of them. In the 12 months following the CCL24 assessment, 29 patients (54.7%) experienced a worsening of FVC, and out of these, 15 (28.3%) experienced at least an absolute 5% deterioration, and 6 (11.3%) experienced a 10-point deterioration. Patients with SSc-ILD with high levels of CCL24 were more likely to experience any worsening (80.0% vs 39.4%, $P = 0.005$) or at least a 5% worsening in the first 12 months (50.0% vs 15.2%, $P = 0.011$) (Figure 2A). Patients who exhibited a relative reduction of 5% and 10% in FVC at 12 months showed higher cases of CCL24, as illustrated in Supplementary Figure 2. Consistent with these findings, there was a moderate correlation between the baseline serum CCL24 level and both the absolute (R = –0.43; $P = 0.001$) (Figure 2B) and relative (R = –0.41; $P = 0.002$) changes in FVC within the first 12-month follow-up period.

Clinical information to define the fulfillment of Erice criteria were available for 61 patients, and PF-ILD was experienced by 27 patients (44.3%). Median serum CCL24 levels at baseline were higher in patients with SSc PF-ILD compared to patients with nonprogressive SSc-ILD, as per the Erice criteria (1,290 [IQR 852–1,800] vs 746 [IQR 532–1,242] pg/mL; $P < 0.05$) (Figure 2C). Consistently, high-CCL24 patients were more likely

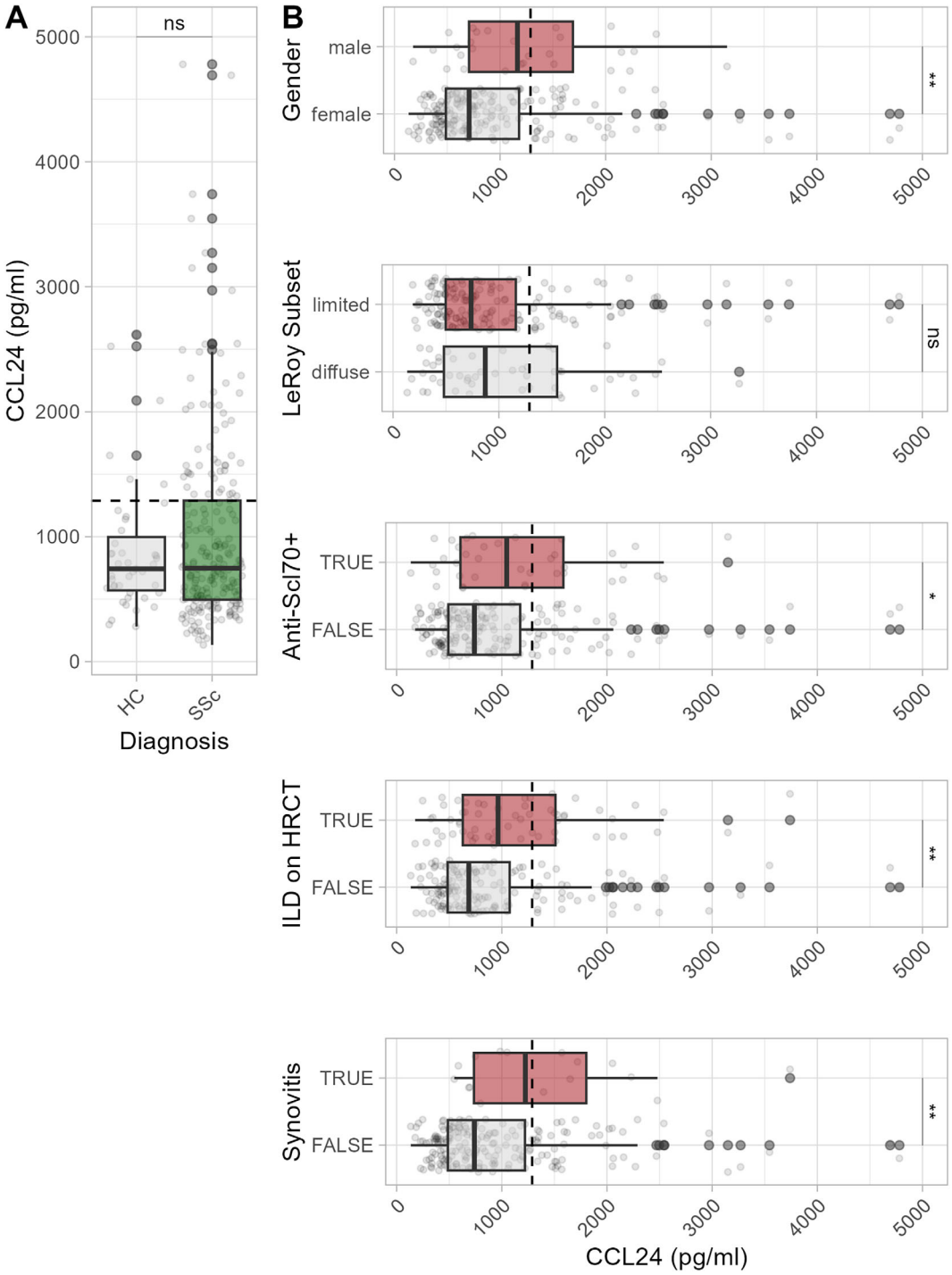


**Figure 1.** Comparison of serum CCL24 levels (A) between patients with SSc and HCs and (B) based on main clinical characteristics. The dotted red lines indicate the threshold for high (Hi) and low (Lo) CCL24 status. Darker dots represent the outliers that are more than 1.5 interquartile below the first or above the third quartile. *$P < 0.05$, **$P < 0.01$. HC, healthy control; Hi, high; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; Lo, low; SSc, systemic sclerosis.

to experience PF-SSc-ILD on follow-up (OR 4.24, 95% CI 1.70–10.5; $P = 0.002$). The relationship between CCL24 and SSc-ILD progression remained statistically significant when CCL24 was treated as a log-transformed continuous predictor. This significance persisted even in analyses that were adjusted for demographic factors (age, sex) and major clinical prognostic factors, including the LeRoy classification and disease duration of three years or less as detailed in the adjusted models reported in the Supplementary Table 2. The comparison of patients with progressive and nonprogressive ILD is reported in Table 2.

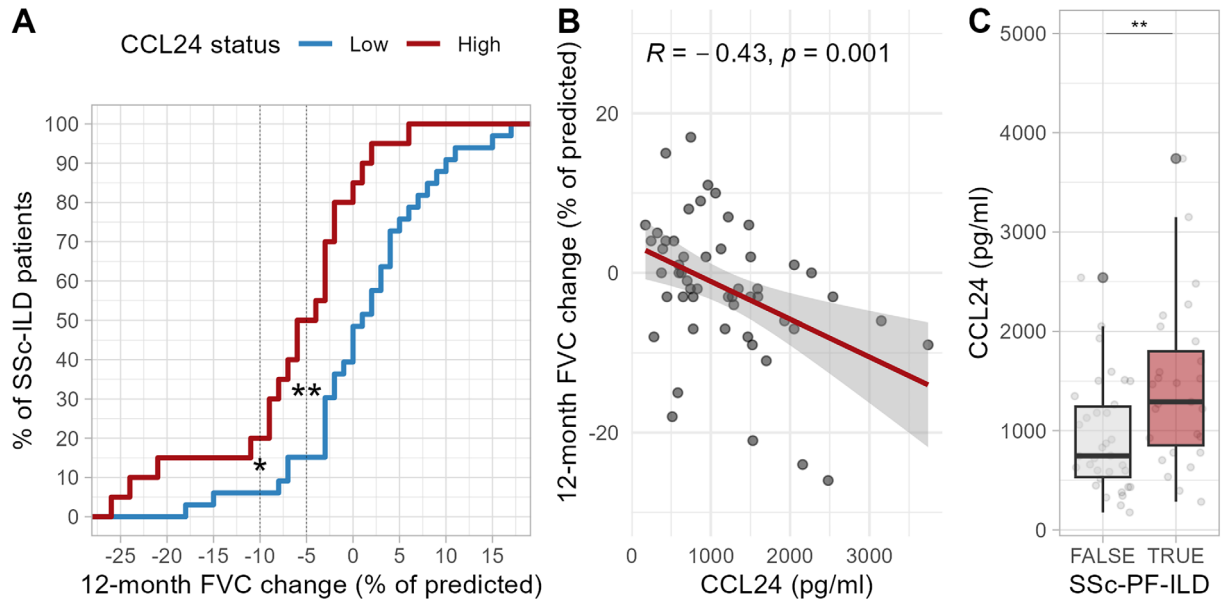


**Figure 2.** Comparison of baseline serum CCL24 levels based on (A) the occurrence of rapid ILD progression, (B) relationship between baseline serum CCL24 levels and the relative change in FVC over the following 12 months, (C) comparison of serum CCL24 levels according to Erice progression criteria in the ILD group. *$P < 0.05$, **$P < 0.01$. FVC, forced vital capacity; ILD, interstitial lung disease; PF-ILD, progressive fibrosing interstitial lung disease; SSc, systemic sclerosis.

Fifty-one patients with the diffuse cutaneous variant were studied, and 12-month mRSS data were available for all. An MCID in mRSS worsening was seen in nine patients. These patients had higher median baseline levels of CCL22 compared to nonprogressors, although not statistically significant (1,263 [IQR 432–1,590] vs 850 [IQR 519–1,529] pg/mL; $P = 0.776$). Similarly, patients with skin progression were more likely to have high baseline CCL24 status than nonprogressors but, again, without statistical significance (44.4% vs 33.3%; $P = 0.804$).

**Serum CCL24 and 10-year mortality.** Follow-up data on mortality were available for all the enrolled patients. A total of 50 deaths were reported over a median follow-up of 6.6 (IQR 5.3–8.9) years, and 20 deaths were classified as SSc-related as the consequence of cardiopulmonary involvement with or without a precipitating infection. The survival distributions were statistically significantly different between high- and low-CCL24 patients ($P = 0.002$), with the first condition associated with a poorer prognosis (Figure 3). Figure 4 presents the separate cumulative incidence of SSc-related and SSc-unrelated deaths in patients with baseline high and low CCL24 status to display the differences according to specific causes of death. Although the cumulative incidence plot of SSc-unrelated death is equivalent between the two groups, patients with high serum levels of CCL24 showed an increased risk of SSc-related deaths. Specifically, a high CCL24 status was associated with a 4-fold increase in the rate of SSc-related death in individuals with SSc (sHR 4.24, 95% CI 1.70–10.50; $P = 0.002$), whereas there was no significant difference in the rate of non–SSc-related deaths. The relationship between CCL24 status and SSc-related mortality remained statistically significant when CCL24 was treated as a log-transformed continuous predictor. This significance persisted even in analyses that were adjusted for demographic factors (age, sex) and major clinical prognostic factors, including the LeRoy classification and disease duration of three years or less as detailed in the adjusted models reported in the Supplementary Table 3.

## DISCUSSION

In this study, we explored the relationship of serum CCL24 levels with SSc severity and prognosis in a real-world cohort of patients to assess its role as a biomarker and as a potential therapeutic target. These associations were examined using CCL24 levels both as a continuous variable and using a pragmatic clinical cutoff derived from the normal distribution in an age- and sex-matched HC group.

The study highlights the association between higher CCL24 levels and critical clinical variables linked to the most severe forms of SSc. Specifically, these variables include male sex, anti–Scl-70 positivity, severity of skin fibrosis, presence of ILD, presence of digital ulcers, and lung microvascular impairment as measured by $DL_{CO}$. In the longitudinal setting, high serum CCL24 was predictive of lung deterioration. Accordingly, a higher baseline CCL24 level was associated with SSc-related mortality.

The association of CCL24 with rapid ILD progression and SSc-related mortality was found to be independent of demographic factors, disease duration, and the LeRoy cutaneous

**Table 2.** General characteristics and CCL24 levels of patients with SSc-ILD and comparison between progressive and nonprogressive patients at 24-month follow-up*

| | | 24-mo ILD evolution | | |
|---|---|---|---|---|
| | Overall | Nonprogressive | Progressive | |
| Clinical variables | N = 61 | N = 34 | N = 27 | *P* value |
| Age, mean ± SD, y | 52.1 ± 13.1 | 50.1 ± 13.3 | 54.7 ± 12.7 | 0.2 |
| BMI, kg/m$^2$, mean ± SD | 27.1 ± 5.5 | 27.3 ± 4.8 | 26.8 ± 6.3 | 0.7 |
| Male sex, n (%) | 18 (29.5) | 9 (26.5) | 9 (33.3) | 0.6 |
| LeRoy diffuse cutaneous variant, n (%) | 35 (57.4) | 17 (50.0) | 18 (66.7) | 0.2 |
| Disease duration, median (IQR), y | 5.0 (3.0–10.0) | 5.0 (3.0–7.8) | 7.0 (3.0–14.5) | 0.4 |
| Disease duration ≤3 y, n (%) | 24 (39.3) | 13 (38.2) | 11 (40.7) | 0.8 |
| Anticentromere positive, n (%) | 7 (11.5) | 4 (11.8) | 3 (11.1) | >0.9 |
| Anti–Scl-70 positive, n (%) | 28 (45.9) | 14 (41.2) | 14 (51.9) | 0.4 |
| Active-late capillaroscopy pattern, n (%) | 35 (71.4) | 20 (71.4) | 15 (71.4) | >0.9 |
| Late capillaroscopy pattern, n (%) | 21 (42.9) | 11 (39.3) | 10 (47.6) | 0.6 |
| mRSS, median (IQR) | 3.0 (2.0–6.0) | 3.5 (1.3–5.0) | 3.0 (2.0–6.0) | 0.4 |
| ILD on HRCT, n (%) | 61 (100.0) | 34 (100.0) | 27 (100.0) | - |
| PAH on RHC, n (%) | 9 (14.8) | 3 (8.8) | 6 (22.2) | 0.2 |
| Baseline FVC, mean ± SD, % of predicted | 90.8 ± 23.2 | 92.6 ± 22.9 | 88.4 ± 23.8 | 0.5 |
| Baseline TLC, mean ± SD, % of predicted | 86.6 ± 19.1 | 82.3 ± 17.4 | 92.1 ± 20.1 | 0.044 |
| Baseline DLco, mean ± SD, % of predicted | 52.7 ± 12.6 | 54.9 ± 12.7 | 50.0 ± 12.1 | 0.13 |
| Baseline FVC ≤80% of predicted, n (%) | 24 (39.3) | 12 (35.3) | 12 (44.4) | 0.5 |
| Baseline DLco ≤50% of predicted, n (%) | 26 (42.6) | 13 (38.2) | 13 (48.1) | 0.4 |
| Baseline FVC ≤80% or DLco ≤50% of predicted, n (%) | 60 (98.4) | 33 (97.1) | 27 (100.0) | >0.9 |
| Digital ulcers (ever), n (%) | 31 (50.8) | 13 (38.2) | 18 (66.7) | 0.027 |
| Digital ulcers (current), n (%) | 8 (13.1) | 4 (11.8) | 4 (14.8) | >0.9 |
| Skin calcinosis, n (%) | 35 (57.4) | 16 (47.1) | 19 (70.4) | 0.067 |
| Skin telangiectasia, n (%) | 37 (60.7) | 18 (52.9) | 19 (70.4) | 0.2 |
| Synovitis (ever), n (%) | 4 (6.6) | 2 (5.9) | 2 (7.4) | >0.9 |
| Myositis (ever), n (%) | 9 (14.8) | 5 (14.7) | 4 (14.8) | >0.9 |
| Immunosuppressive treatment, n (%) | 37 (60.7) | 17 (50.0) | 20 (74.1) | 0.056 |
| Vasoactive treatment, n (%) | 36 (59.0) | 18 (52.9) | 18 (66.7) | 0.3 |
| CCL24, median (IQR), pg/mL | 964.0 (629–1,510) | 746.0 (531–1,242) | 1,290 (852–1,800) | 0.006 |
| Ln CCL24, mean ± SD | 6.9 ± 0.7 | 6.7 ± 0.6 | 7.1 ± 0.6 | 0.006 |
| Hi CCL24, n (%) | 22 (36.1) | 8 (23.5) | 14 (51.9) | 0.022 |

* BMI, body mass index; DLco, diffusing capacity of the lung for carbon monoxide; Hi, high; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; FVC, forced vital capacity; IQR, interquartile range; Lo, low; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; RHC, right heart catheterization; TLC, total lung capacity.

variant, highlighting its prognostic value over traditional clinical prognostic factors. In the subgroup of patients with diffuse cutaneous variant, no statistically significant association was found between CCL24 and the progression of skin fibrosis during follow-up. This result might be influenced by the limited sample size and a substantial proportion of patients with long-standing SSc, in whom skin fibrosis progression is generally less likely compared to those in the early stages of the disease.

Our data support the inclusion of CCL24 as a serological biomarker for disease activity and ILD progression both in clinical management and as an enrichment strategy in clinical trials. The reported correlations align with preclinical evidence of the activity of CCL24 and the biologic characterization of samples from patients with SSc. The association of serum CCL24 levels with mRSS is consistent with the observed hyperexpression in skin biopsy samples from patients with diffuse SSc.[5] Similarly, the association of CCL24 levels with ILD presence and prognosis aligns with the ability of CCL24 to induce lung fibroblast proliferation in vitro,[16] reduced infiltration of immune cells in bleomycin induced model in mice lacking the CCL24 gene, and the inhibitory effect of CCL24 blockade on lung fibrosis with the reduction of immune cell infiltration in BALF in murine model.[5] Notably, although CCL24 has not been assessed in vivo in lung specimens from patients with SSc-ILD, CCL24 levels were found to be higher in the BALF of patients with idiopathic pulmonary fibrosis compared to controls.[17] Lastly, although synovitis is not common in SSc, the association of this condition with high CCL24 could reflect the protective effect of its inhibition in adjuvant-induced arthritis.[18]

CCL24 is a chemokine that promotes cell trafficking and regulates profibrotic and inflammatory activities through the CCR3 receptor. It was originally discovered as a chemokine inducing immune cell trafficking, primarily eosinophils and basophils,[4] and subsequently it was recognized as a pleiotropic cytokine directly affecting monocytes migration, macrophage polarization, fibrosis, and endothelial function.[19,20] Because a

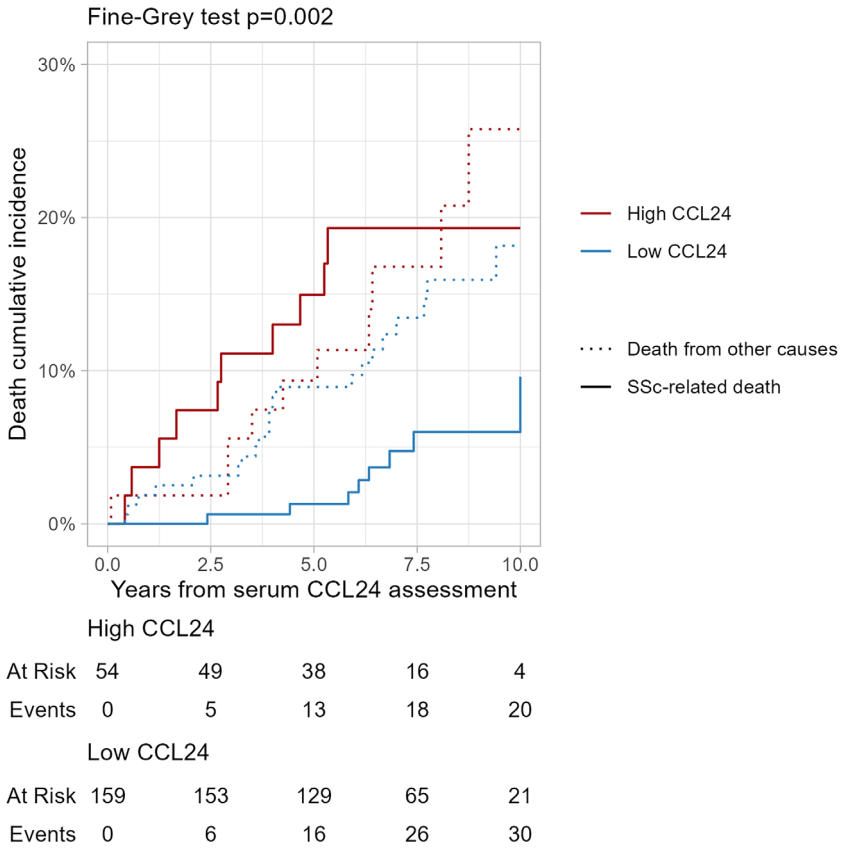


**Figure 3.** Survival curves comparing baseline high and low CCL24 patient raw mortality. High CCL24 status was defined as serum values exceeding 1,288 pg/mL. SSc, systemic sclerosis.

direct pathophysiologic role of eosinophils in SSc has not been demonstrated, it can be hypothesized that the relationship of eosinophil counts with disease severity and prognosis could represent an epiphenomenon of the multiple biologic activities of CCL24. Consistent with this hypothesis, a higher peripheral eosinophil count has been associated with the severity of cutaneous and pulmonary fibrotic involvement,[21] as well as the presence and recurrence of digital ulcers over time.[22] Furthermore, BALF eosinophilia has been repeatedly associated with the severity of parenchymal involvement and mortality in patients with SSc-ILD.

The evidence from animal models and the clinical findings reported support the notion of CCL24 as a therapeutic target for SSc. Given its association with both vascular and fibrotic manifestations of the disease, targeting CCL24 could provide clinical benefits across limited and diffuse cutaneous subsets and help manage simultaneously the heterogeneous clinical manifestations of the disease.

Of note, patients with high CCL24 were more likely to be treated with immunosuppressants compared to those with low CCL24 in our real-life cohort of patients with SSc. This observation, on the one hand, reflects the practice of using immunosuppressants in the most severe form of the disease but, on the other, indicates that currently available medications are unlikely to adequately impact CCL24 levels. Therefore, there could be therapeutic potential for CCL24 inhibition as a complementary strategy to manage disease activity despite current standard of care, particularly to improve the prognosis of high-risk patients.

This study has some limitations, including its retrospective design, the relatively small sample size that did not allow for multivariate adjustments, and the absence of validation in an external independent cohort, which could limit the generalizability of the reported findings. We particularly acknowledge that the number of healthy controls analyzed in this study is relatively low and could not allow a proper determination of "upper limit of normal" as far as CCL24 serum concentration. To mitigate for this limitation, we adopted a conservative approach and categorized patients simply one SD over the mean log-transformed concentration. Finally, the population analyzed did not allow a proper analysis of skin progression given the long disease duration. A tailored study on early diffuse cutaneous SSc is warranted to determine whether CCL24 serum concentration predicts SSc skin progression as we have shown for SSc-ILD.

CCL24 levels reflect disease severity in this real-world cohort of patients with SSc and are associated with both fibrotic and vascular complications. Higher serum CCL24 levels are associated with an increased risk of progression and severity in patients

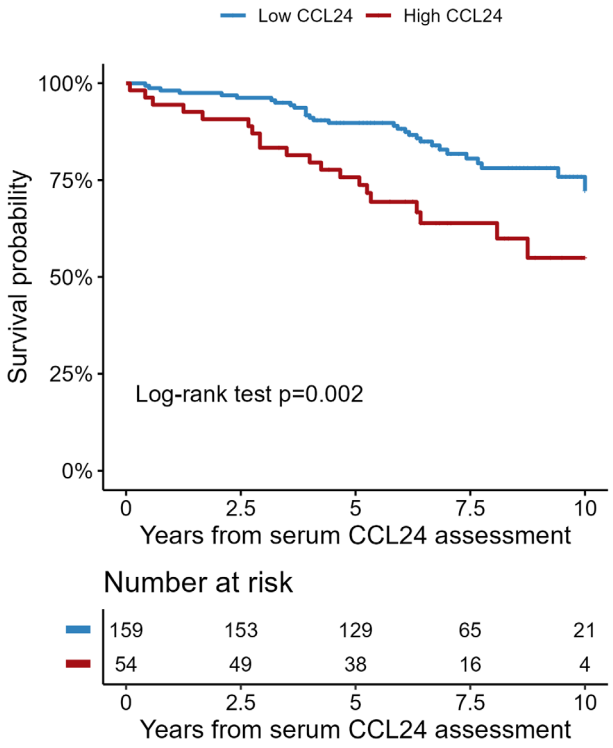


**Figure 4.** Cumulative incidence curves comparing SSc-related and other-cause mortality in SSc patients with High and Low baseline CCL24 Status. High CCL24 status was defined as serum values exceeding 1,288 pg/mL. SSc, systemic sclerosis.

with SSc-associated ILD. Furthermore, higher serum CCL24 levels are associated with an elevated SSc-related death rate. These findings support the potential involvement of CCL24 in the pathophysiology of SSc and underscore its potential as a promising therapeutic target for 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. FDG 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.** Mor, Del Galdo.
**Acquisition of data.** De Lorenzis, Mor, Ross, Di Donato, Del Galdo.
**Analysis and interpretation of data.** De Lorenzis, Aricha, Vaknin, Del Galdo.

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