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Original research

Mediators of systemic sclerosis-associated interstitial lung disease (SSc-ILD): systematic review and meta-analyses

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► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/thorax-2022-219226>).

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Received 23 May 2022
Accepted 23 September 2022
Published Online First
19 October 2022



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To cite: Fields A, Potel KN, Cabuhal R, et al. *Thorax* 2023;**78**:799–807.

ABSTRACT

Systemic sclerosis-associated interstitial lung disease (SSc-ILD) is rare, poorly understood, with heterogeneous characteristics resulting in difficult diagnosis. We aimed to systematically review evidence of soluble markers in peripheral blood or bronchoalveolar lavage fluid (BALF) as biomarkers in SSc-ILD.

Method Five databases were screened for observational or interventional, peer-reviewed studies in adults published between January 2000 and September 2021 that assessed levels of biomarkers in peripheral blood or BALF of SSc-ILD patients compared with healthy controls. Qualitative assessment was performed using Critical Appraisal Skills Programme (CASP) checklists. Standardised mean difference (SMD) in biomarkers were combined in random-effects meta-analyses where multiple independent studies reported quantitative data.

Results 768 published studies were identified; 38 articles were included in the qualitative synthesis. Thirteen studies were included in the meta-analyses representing three biomarkers: KL6, SP-D and IL-8. Greater IL-8 levels were associated with SSc-ILD in both peripheral blood and BALF, overall SMD 0.88 (95% CI 0.61 to 1.15; $I^2=1\%$). Greater levels of SP-D and KL-6 were both estimated in SSc-ILD peripheral blood compared with healthy controls, at an SMD of 1.78 (95% CI 1.50 to 2.17; $I^2=8\%$) and 1.66 (95% CI 1.17 to 2.14; $I^2=76\%$), respectively.

Conclusion We provide robust evidence that KL-6, SP-D and IL-8 have the potential to serve as reliable biomarkers in blood/BALF for supporting the diagnosis of SSc-ILD. However, while several other biomarkers have been proposed, the evidence of their independent value in diagnosis and prognosis is currently lacking and needs further investigation.

PROSPERO registration number CRD42021282452.

INTRODUCTION

Systemic sclerosis (SSc) is a rare rheumatic disease with heterogeneous characteristics, which pose challenges for accurate diagnosis and prognosis. Scleroderma (skin thickening) is a common symptom of SSc, often preceded by Raynaud's phenomenon. Patients can develop organ involvement, such as interstitial lung disease (ILD) that can lead to progressive lung fibrosis. SSc-associated interstitial lung disease (SSc-ILD) and its attributed decline in lung function is a primary cause of death in patients with SSc.¹ Furthermore, diagnosis of

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Systemic sclerosis-associated interstitial lung disease (SSc-ILD) is rare, poorly understood, with heterogeneous characteristics resulting in difficult diagnosis. We aimed to systematically review evidence of soluble markers in peripheral blood or bronchoalveolar lavage fluid (BALF) as biomarkers in SSc-ILD.

WHAT THIS STUDY ADDS

⇒ Screening (January 2000–September 2021) across five databases identified 768 publications for systematic review of soluble biomarkers in SSc-ILD. A total of 38 studies were included in qualitative review; 13 studies were included in random effects meta-analysis providing quantitative synthesis on KL6, SP-D and IL-8.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our systematic review provides robust evidence that high IL-8 in blood and BALF, and higher blood SP-D and KL6 were associated with SSc-ILD, supporting earlier disease management and potential theranostics. However, while several other biomarkers have been reported including those mediators stated in peripheral blood and BALF, the evidence of their independent value in diagnosis and prognosis is currently lacking and needs further investigation.

pleuroparenchymal fibroelastosis (PPFE) is not uncommon in SSc-ILD and is associated with worse prognosis.²

There are currently very limited treatments specific for SSc-ILD.³ Most patients are either monitored without intervention or receive immunosuppressive treatment. Antifibrotics can slow down lung function decline in progressive lung fibrosis and have been approved by the Food and Drug Administration (FDA) for SSc-ILD in the USA⁴ and recently by the National Institute for Health and Care Excellence in the UK.⁵ The humanised interleukin-6 (IL-6) receptor antagonist tocilizumab has recently been approved by the FDA after showing to reduce progressive loss of lung function for patients with SSc-ILD compared with placebo (focuSSced trial).⁶ Nevertheless, timely diagnosis of ILD in SSc

continues to be a challenge. The current diagnostic gold standard is high-resolution CT (HRCT) scans, with the most prevalent histopathological/radiographical pattern being non-specific interstitial pneumonia,^{7,8} although monitoring ILD development exposes individuals with SSc to frequent irradiation. Lung biopsies have been used to investigate the lung architecture of those with SSc-ILD, however, this invasive procedure would not be indicated for regular use. For many years, diagnosis was based on pulmonary function tests (PFTs); however, over 60% of patients diagnosed with SSc-ILD may have normal PFT ranges.⁹ To support early ILD management, there is a need for new methods of early diagnosis, which may include the detection of soluble mediators.

Numerous soluble systemic mediators (whole blood, serum and/or plasma) have been identified in patients with SSc-ILD compared with healthy groups or other disease states. Further characterisation of systemic mediators may enable a more straightforward method of diagnosis, in addition to providing an accessible and quantitative method of monitoring disease. An alternative source of soluble mediators may be detected within bronchoalveolar lavage fluid (BALF). As a more invasive procedure, repeated performance of BAL techniques for the measurement of soluble markers to monitor organ-specific disease severity and progression may not be a viable resource but may complement decision making where ILD needs further confirmation and characterisation.

However, consideration of both blood and BALF markers in isolation and combination could provide insights for the identification of new pharmaceutical targets for patients with SSc-ILD and improvement of preclinical models, yet at present there are no accepted disease-defining biomarkers. We conducted a systematic review to identify soluble markers in blood and BALF

as potential candidates for a screening panel for SSc-ILD, and to explore possible pathways for intervention.

METHODS

Search methods

Extensive searches were performed on Embase, Web of Science, Medline ALL, Scopus and PubMed for papers published from 1 January 2000 to 30 September 2021. Searches were not restricted by language or study design. Review articles were excluded through EndNote V.10 search. Searches were last updated in October 2021. Further information can be found in online supplemental file 1. The prespecified systematic review protocol was made available through a prospective public register (PROSPERO, CRD42021282452). Throughout the review, PRISMA guidelines¹⁰ were followed.

Selection criteria

Published, peer-reviewed papers were considered which were prospective or retrospective, case-control or cohort studies of any size. Articles for this review were selected based on their relevance to the analyses of soluble mediators and biomarkers in SSc-ILD. Eligible studies contained samples collected from any population of adults diagnosed with SSc-ILD, evidenced by clinical features, chest HRCT and/or lung biopsy, and were included regardless of immunosuppressive treatment. Included studies investigated soluble mediators evaluated in blood (serum/plasma) and BALF in subjects diagnosed with SSc-ILD compared with healthy controls (figure 1).

Data collection

Data were manually extracted from retrieved full-text versions of included studies, and data were confirmed by a second

Search Terms: “systemic sclerosis” AND “interstitial lung disease” AND biomarker AND (plasma OR serum OR blood OR “whole blood”) OR (“systemic sclerosis” AND “interstitial lung disease” AND biomarker AND (“lung lavage” OR “bronchoalveolar lavage”).
Search time-period: 1st January 2000 – 30th September 2021

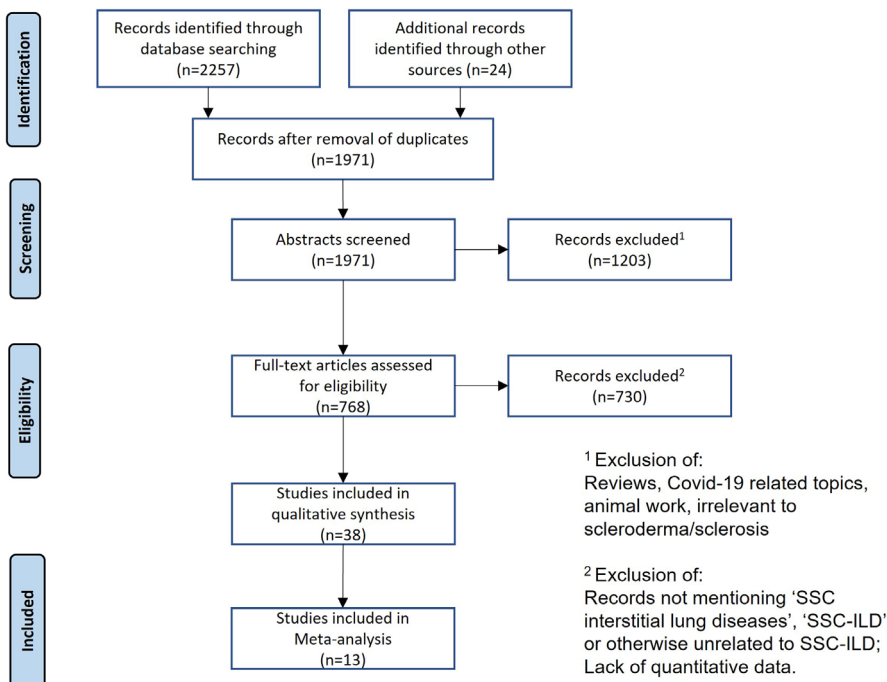


Figure 1 PRISMA flow diagram.¹⁰ SSc-ILD, systemic sclerosis-associated interstitial lung disease.

author. Only data regarding soluble mediators were retrieved; miRNA expression data, cellular information and exhaled nitric oxide data were not included. All data were available from the reviewed articles. Baseline data were retrieved from longitudinal studies. Where statistical analyses of the data were only presented as scatter plots, data description (mean/median, SD/IQR/range) was estimated from the image. Study information including biomarker levels, units, sample sizes and treatment, were extracted and reported, and data were presented as given in the published articles.

When descriptive data were missing for patients or controls (BALF: IL-12¹¹ IL-8, CxCL5 and S100A8/A9¹²; peripheral blood: selectins,¹³ calpain and HMGB-1,¹⁴ CX3CL1 (fractalkine),¹⁵ gremlin-1,¹⁶ KL-6,^{12 17} osteopontin (OPN),¹⁸ SP-D¹⁷ and IL-8,¹² the authors were contacted. Where we did not receive a response from the authors, data were estimated from presented graphs using <https://automeris.io/WebPlotDigitizer/>.

The overall quality of the included evidence and risk of bias (RoB) was assessed by two independent assessors (KNP and BCS) using the Critical Appraisal Skills Programme (CASP) Checklist for Cohort Studies.¹⁹ Papers were assessed using ten predefined questions with regards to domains of 'Study Design and Methodology', 'Measurements and Results' and 'External Validity' including study purpose, patient population, methods and data analysis (online supplemental file 2). Individual questions were scored: 1=appropriate, 0=uncertain, -1=inappropriate or incomplete. Scores for the individual questions were summarised in an overall weighted RoB score ranging from -30 (very high RoB) to 30 (very low RoB). The judgements on overall quality were discussed and confirmed by KNP and BCS.

Data analyses

Statistical analysis

Where a specific marker was represented by more than two independent studies, standardised mean difference (SMD) was used to obtain overall effect sizes in a random effects model.^{20 21} Medians and IQRs were transformed into estimated means and standard deviations.²² Q test assessed heterogeneity between studies and was presented via the inconsistency index (I^2) as a percentage. The proportion of total variability due to between-study heterogeneity was classified as low (<25%), moderate (25%–50%), large (50%–75%) or very large (>75%).²³ Where heterogeneity was considered large, sensitivity analyses were performed to remove outliers. Where possible, subgroup analyses were performed according to sample source and methodology of quantitation. All analyses were performed with Review Manager software (RevMan, V.5.4, The Cochrane Collaboration, 2020).

Pathway analysis

To explore pathways and biological processes involved in SSc-ILD that could be identified through this review, genes encoding biomarkers included in the meta-analysis and those present in BALF, and peripheral blood were compiled in g:Profiler²⁴ using Ensembl IDs of identified markers and over-representation of information from known biological pathways (gene ontology enrichment analysis for biological processes, Go:BP) and protein–protein networks (string analysis) were described. Correction for multiple testing was performed using Bonferroni.²⁵ Ensembl IDs used can be found in online supplemental file 3.

RESULTS

A total of 2281 citations were retrieved, of which 1971 unique abstracts were identified through searching of the five databases, and by manual searching of review articles and additional reading. Screening of these abstracts led to the inclusion of 768 publications for full-text review, out of which 730 publications were ineligible. A total of 38 publications were considered eligible for inclusion in the review, with thirteen included in the meta-analysis (presented in the PRISMA flow diagram¹⁰ (figure 1). Evidence was mostly based on observational studies (n=36; 95%); two studies were intervention studies.

Further extracted study information can be found in online supplemental file 4. 24% of studies excluded patients receiving any form of immunosuppressive treatment or stated that only treatment naïve patients were included. 32% of studies explicitly excluded patients with relevant comorbidities, for example, autoimmune, overlapping syndromes such as sarcoidosis and other lung diseases, and 42% of studies looked at mediator level correlation with clinical progression to assess prognostic biomarker potential (online supplemental file 1).

Quality of the evidence and RoB assessment

The RoB for each study was evaluated using a 10-point checklist that focused on key elements of internal and external validity (online supplemental file 2). Most studies reported reproducible and comparable methods of patient recruitment, SSc-ILD diagnosis, as well as means of measuring and analysing mediator levels. In many cases, the external validity was difficult to assess due to a lack of comparable studies that could either support or challenge reported results. The overall RoB was low among the 38 studies included in the qualitative analysis (figure 2).

All studies (38/38; 100%) were assessed as having a low RoB in the domain of 'study design and methodology'. A total of six studies (15.8%) were assessed as having a moderate RoB in the domain 'measurements and results', while the remaining 32 (84.2%) had a low RoB. The majority of studies (36/38; 94.7%) were assessed as having a low RoB in domain of 'external validity', while 1 had a moderate risk (2.6%) and one a had high risk (2.6%). The overall weighted RoB was low in 37/38 studies (97.4%), and moderate in 1/38 studies (2.6%) (figure 3).

Systemic and pulmonary markers in SSc-ILD

The identified markers were divided into two categories: We identified forty-three secreted proteins in peripheral blood (online supplemental file 5) and fifteen mediators in BALF (online supplemental file 6).

In blood, 5 out of the 43 markers (11.6%) were reported to be significantly lower in patients with SSc-ILD compared with healthy controls (Von Willebrand factor-cleaving protease (Adams-TS),²⁶ insulin-like growth factor 1 (IGF-1),²⁷ cathelicidin antimicrobial peptide (LL-37, CAMP),²⁸ adhesion molecule L-selectin¹³). All other mediators (online supplemental file 5) were significantly increased in peripheral blood of patients with SSc-ILD. Within peripheral blood markers, Krebs von den Lungen (KL)-6, surfactant protein (SP)-D and interleukin (IL)-8 were most represented across the eligible studies, with eight studies providing data on KL-6 concentrations, 6 studies on SP-D and 3 studies on IL-8 blood levels. Only two studies each were identified on blood CCL18 and IL-6 levels in SSc-ILD (online supplemental file 5).

In BALF, all identified markers were increased in concentration compared with healthy controls (online supplemental file 6). Significantly increased levels of IL-8 in BALF were found in

	Study Design and Methodology	Measurements and Results	External Validity	Total
Andersen et al, 2007	20	10	15	45
Benfante et al, 2018	20	20	20	60
Bonella et al, 2011	20	20	20	60
d'Alessandro et al, 2021	18	20	20	58
De Santis et al, 2011	20	20	15	55
Gao et al, 2020	18	13.34	15	46.34
Gedik et al, 2020	20	16.67	15	51.67
Grosicka et al, 2018	20	13.34	20	53.34
Guiot et al, 2021	20	20	15	55
Habe et al, 2017	20	16.67	15	51.67
Hant et al, 2009	20	16.67	20	56.67
Hasegawa et al, 2014	20	10	15	45
Hesselstrand et al, 2013	20	13.34	15	48.34
Hizal et al, 2015	18	20	15	53
Hoffmann-Vold et al, 2018	20	10	15	45
Iannone et al, 2021	20	16.67	15	51.67
Kennedy et al, 2015	20	10	15	45
Khadilkar et al, 2019	16	16.67	5	37.67
Kuzumi et al, 2021	20	16.67	15	51.67
Lee et al, 2012	18	10	15	43
Mathai et al, 2010	18	10	15	43
Meloni et al, 2004	18	13.34	15	46.34
O'Reilly, 2021	18	10	15	43
Sakamoto et al, 2015	20	20	20	60
Salazar et al, 2018	20	20	20	60
Sawicka et al, 2017	18	16.67	15	49.67
Schmidt et al, 2009	18	20	15	53
Storkanova et al, 2021	20	16.67	15	51.67
Takahashi et al, 2000	20	20	15	55
Taniguchi et al, 2018	20	16.67	15	45
Volkman et al, 2019	20	20	15	60
Volkman et al, 2016	18	20	20	60
Wakhlu et al, 2018	20	16.67	20	58
Yanaba et al, 2004	18	13.34	20	55
Yanaba et al, 2013	20	10	15	46.34
Zhang et al, 2020	20	20	15	51.67
Zheng et al, 2020	20	10	15	53.34
Zolkiewicz et al, 2020	20	16.67	20	55

Figure 2 Risk of bias assessment summary of studies included in the qualitative analyses. Studies were scored (0, 1 or 2) on these study domains: 1. Study Design and Methodology (5 questions, weighing × 2, maximum 20); 2. Measurements and results (3 questions, weighing × 3.3, maximum 20) and external validity (2 questions, weighing × 5, maximum 20). The individual study domain assessments scored 0–20. Overall scoring 41–60=green, 21–40=orange, 0–20=red. SSc-ILD, systemic sclerosis-associated interstitial lung disease.

four studies, but all other BALF markers were represented in single studies. For BALF IL-10 and IL-12, measurable concentrations were identified only in patients with SSc-ILD. In healthy controls both cytokines were below the limit of detection.¹¹

Six markers were found to be increased in both blood and BALF samples: CCL2, IL-8, IL-10, human epididymis protein (HE4), alpha-defensin (HNP1) and MMP-9. TNF-alpha was also significantly modified in both sample types. However, while

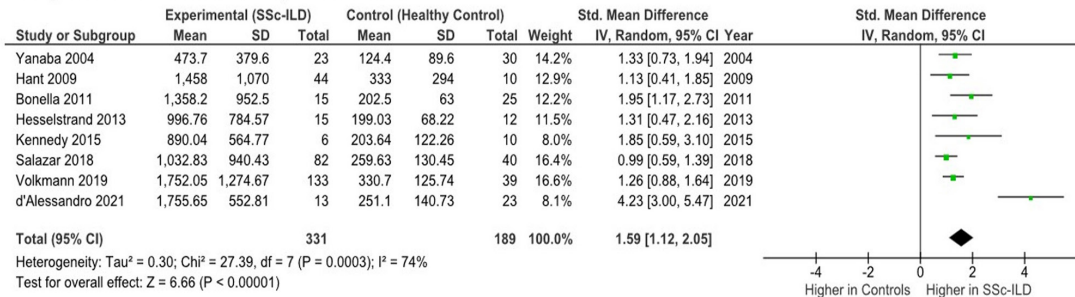
it was increased in SSc-ILD BALF,²⁹ systemic levels (peripheral blood) were reported to be reduced in SSc-ILD.³⁰

Meta-analyses of identified markers

Identified markers represented in more than two eligible, independent studies were included in the meta-analyses. For KL-6, eight publications, which reported plasma/serum levels in a total

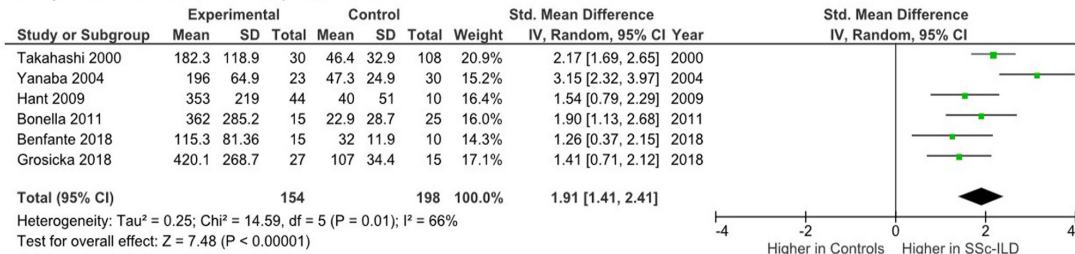
A

Comparison: SSc-ILD vs. Control, Outcome KL-6



B

Comparison: SSc-ILD vs. Control, Outcome SP-D



C

Comparison: SSc-ILD vs. Control, Outcome IL-8

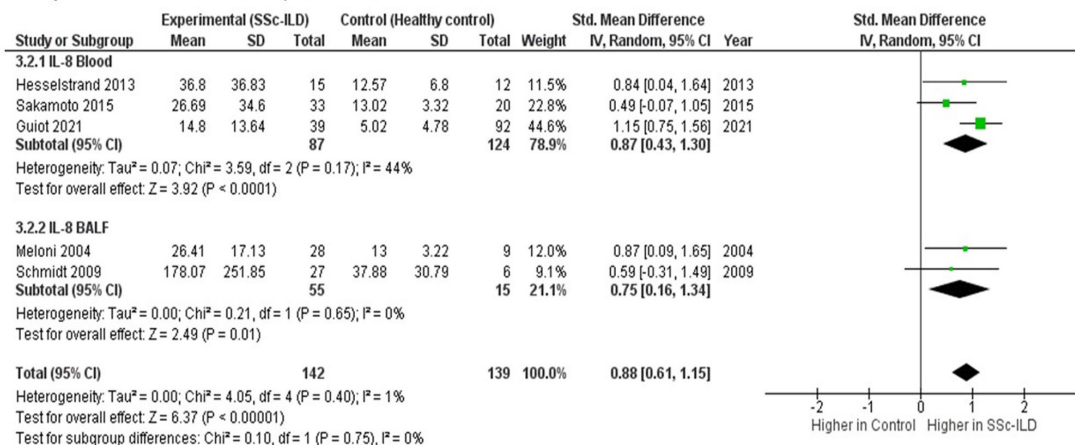


Figure 3 3 Meta-analyses and forest plots of soluble mediators from patients with SSc-ILD compared with healthy controls: (A) KL-6, (B) SP-D and (C) IL-8. SSc-ILD, systemic sclerosis-associated interstitial lung disease.

of 331 SSc-ILD patients and 189 healthy controls, were included in the meta-analysis (figure 3A). The SMD of KL-6 levels in SSc-ILD compared with healthy controls was 1.66 (95% CI 1.17 to 2.14). Variability attributed to between-study heterogeneity of KL-6 effect sizes was very large (I²: 74%). Sensitivity analysis suggested that one study mainly contributed to this heterogeneity. Exclusion of the 13 patients and 23 healthy controls measured by d'Alessandro *et al*³¹ reduced heterogeneity to low (I²: 0%) while not affecting the direction of estimate (standard mean difference changed from 1.59 (95% CI 1.12 to 2.05) to 1.25 (95% CI 1.04 to 1.47),; online supplemental file 5B). The sensitivity owing to different methodologies reported to quantify the analytes KL-6, Sp-D and IL-8 in peripheral blood (online supplemental file 5C), subgroup analyses were performed according to the different methods and suggested similar effect estimates (online supplemental file 5B and 5D).

Serum SP-D was reported in six studies, measuring levels within plasma/serum of 154 patients with SSc-ILD and 198 healthy controls. The SMD in SSc-ILD patients compared with healthy controls was 1.91 (95% CI 1.41 to 2.41), with high heterogeneity observed (I²=66%) (figure 3B). Exclusion of the 23 patients and 30 healthy controls measured by Yanaba *et al*¹⁷ reduced heterogeneity to low (I²: 0%) while not affecting the direction of estimate (SMD changed from 1.91 (95% CI 1.41 to 2.41) to 1.47 (95% CI 1.38 to 2.10), online supplemental file 5E).

IL-8 levels were reported in plasma/serum and BALF. We identified three suitable studies reporting plasma/serum levels of IL-8 in a total of 87 SSc-ILD patients and 124 healthy controls. In addition, another two independent studies of IL-8 in BALF, representing a total of 55 SSc-ILD patients and 15 healthy controls, were identified and included. Overall, the SMD for

Table 1 Top 20 most enriched pathways based on adjusted p value

Term ID	Description	P adj	Hit count	Mediators (hits) involved in pathway								
				MMP-9	HNP1	CCL2	HE4	IL-10	IL-8	KL-6	SP-D	TNFa
GO:0050900	Leucocyte migration	3.59E-08	7	x	x	x		x	x		x	x
GO:0006959	Humoral immune response	1.48E-06	6		x	x	x		x		x	x
GO:0009617	Response to bacterium	3.87E-06	7		x	x	x	x	x		x	x
GO:0071222	Cellular response to lipopolysaccharide	1.82E-05	5		x	x		x	x			x
GO:0071219	Cellular response to molecule of bacterial origin	2.25E-05	5		x	x		x	x			x
GO:0006952	Defence response	2.39E-05	8	x	x	x	x	x	x		x	x
GO:0030595	Leucocyte chemotaxis	3.44E-05	5		x	x		x	x		x	
GO:0071216	Cellular response to biotic stimulus	4.15E-05	5		x	x		x	x			x
GO:0042742	Defence response to bacterium	1.28E-04	5		x		x	x			x	x
GO:0060326	Cell chemotaxis	1.37E-04	5		x	x		x	x		x	
GO:0032496	Response to lipopolysaccharide	1.82E-04	5		x	x		x	x			x
GO:0030155	Regulation of cell adhesion	2.29E-04	6			x		x	x	x	x	x
GO:0019730	Antimicrobial humoral response	2.40E-04	4		x		x		x		x	
GO:0002237	Response to molecule of bacterial origin	2.40E-04	5		x	x		x	x			x
GO:0006950	Response to stress	3.29E-04	9	x	x	x	x	x	x	x	x	x
GO:1904707	Positive regulation of vascular associated smooth muscle cell proliferation	3.32E-04	3	x				x				x
GO:0016477	Cell migration	4.01E-04	7	x	x	x		x	x		x	x
GO:0051707	Response to other organism	4.71E-04	7		x	x	x	x	x		x	x
GO:0043207	Response to external biotic stimulus	4.77E-04	7		x	x	x	x	x		x	x
GO:0009607	Response to biotic stimulus	5.68E-04	7		x	x	x	x	x		x	x
Total				5	18	17	9	18	18	2	14	17

Also shown are how many genes (hit count) and which genes (mediators involved in pathway) in the query list are involved in each pathway.

IL-8 in SSc-ILD compared with healthy controls was 0.88 (95% CI 0.61 to 1.11) with low heterogeneity ($I^2=1\%$). SMD was similar between serum/plasma (0.87; 95% CI 0.43 to 1.30) and BALF estimates (0.75; 95% CI 0.16 to 1.34) in subgroup analysis (figure 3C). To determine IL-8 in BALF, Schmidt *et al*²⁹ used a multiplex method, while no information was given by Meloni *et al*,¹¹. We therefore performed a sensitivity analysis. However, exclusion of any one of these studies did not change the heterogeneity ($I^2: 0\%$) or the direction of the estimate (SMD changed from 0.72 (95% CI 0.32 to 1.12) to 0.75 (95% CI 0.31 to 1.19) and to 0.67 (95% CI 0.21 to 1.13) with the exclusion of the study by Schmidt *et al*²⁹ and Meloni *et al*,¹¹ respectively (online supplemental file 6C).

The GO analyses for the enrichment of biological processes (GO:BP, table 1) and protein-protein association network analysis (string analysis, online supplemental file 7) of soluble markers from the meta-analysis and those found to be present in both peripheral blood and BALF identified through this review are involved in pathways strongly related to cytokine and chemokine signalling, emphasising the strong associations with a dysregulated immune response (particularly the innate response) in SSc-ILD (figure 4).

DISCUSSION

Overall, the identified SSc-ILD mediators present in peripheral blood or BALF of patients with SSc-ILD can be grouped into alveolar epithelial proteins, mediators of cytokine and chemokine signalling and ECM remodelling mediators. Meta-analyses were performed to explore the effect of selected mediators on SSc-ILD where multiple studies with quantitative data were identified.

Our systematically synthesised data on soluble markers strongly indicated a significant increase in the overall mean difference of KL-6, SP-D and IL-8 in SSc-ILD, providing robust evidence from independent studies for the utility of these biomarkers in diagnosis, particularly in peripheral blood. Intriguingly, to our knowledge, this review for the first time reports secreted mediators that were differentially expressed in SSc-ILD in studies from both peripheral blood and BALF (CCL2, IL-8, IL-10, HE4, HNP and MMP-9).

KL-6 is a mucin-like glycoprotein derived from type 2 alveolar epithelial cell surface³² that regulates expression of collagen and myofibroblast differentiation.³³ KL-6 concentration is found to correlate with FVC,^{34,35} diffusing capacity of lung for carbon monoxide, as well as the extent of lung fibrosis³⁶ and disease progression,³⁷ suggesting KL-6 as a potential prognostic biomarker for SSc-ILD. Furthermore, possible cut-off values to discriminate between SSc-ILD and healthy individuals, SSc without ILD and those with SSc who are at greater or lower risk of progression of SSc-ILD have been suggested.^{36,38-40} Moreover, in patients with SSc-ILD who also showed evidence of PPFE, KL-6 levels may be a useful biomarker of disease progression.³¹ We estimated a greater level of KL-6 in SSc-ILD compared with healthy controls in meta-analyses of eight studies.

Circulating concentrations of SP-D (and SP-A), secreted by alveolar type 2 cells, are seen to reflect the extent of damage to alveolar-capillary barriers.⁴¹ However, SP-A and SP-D in peripheral blood are not specific to SSc-ILD.⁴² Surfactant proteins and surfactant lipids also have anti-inflammatory effects and surfactant lipids (phosphatidyl choline (PC) and phosphatidyl glycerol (PG)) may protect against fibrogenesis by inducing fibroblast

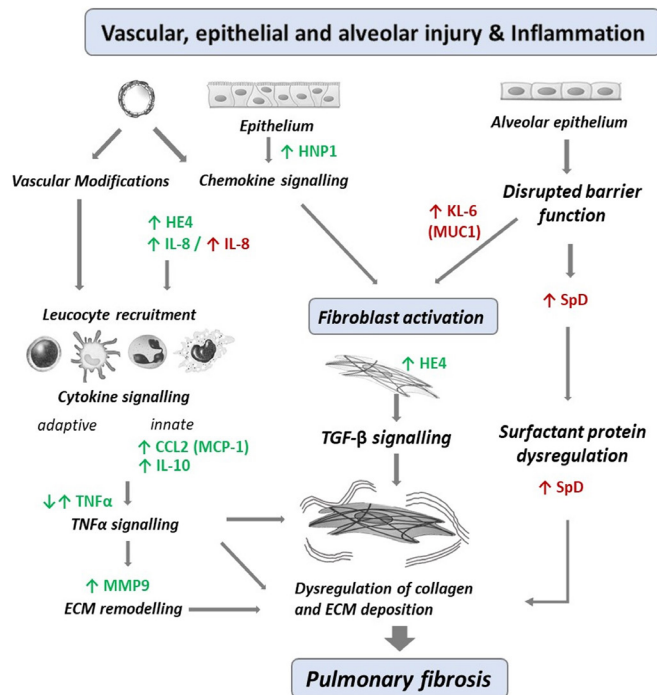


Figure 4 Interaction of pathways and soluble mediators in the pathogenesis of pulmonary fibrosis in SSc-ILD. Green text represents significant markers identified in blood and BALF, while red text represents mediators from the meta-analyses. Adapted from Martens *et al*⁵⁶ with inclusion of pathways identified by g:Profiler.²⁵ BALF, bronchoalveolar lavage fluid; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

apoptosis and decreasing collagen accumulation.⁴³ SP-D and SP-A showed significant differences between SSc patients who were ILD positive or negative, and SP-D has been regarded to have more clinical potential.⁴⁴ We estimated a greater level of SP-D in SSc-ILD across four studies compared with healthy controls, with low heterogeneity.

Cytokines and chemokines contribute to the dysregulation of tissue repair in SSc-ILD through their interactions with fibroblasts and immune cells; however, the mechanisms underlying the recruitment and activation of fibroblasts in SSc-ILD remain incompletely understood.⁴⁵ On an inflammatory stimulus, peripheral blood cells such as macrophages, the airway epithelium and endothelium release IL-8, a well-described chemokine attracting neutrophils to the site of injury. IL-8 is found in both blood and BALF, though concentrations were far higher in BALF. SSc-ILD BALF typically shows an increased number of granulocytes, especially neutrophils.^{46 47} Recently, a higher neutrophil count in patients SSc-ILD has been found to be associated with a worse disease course and higher long-term mortality,⁴⁸ similar to earlier findings in IPF where BALF neutrophils were identified as an independent predictor of early mortality.⁴⁹ Contrasting conclusions have been reported concerning the correlation of BALF IL-8 with lung function.^{29 50} Our meta-analysis identified increased levels of IL-8 in SSc-ILD and suggested similar standardised effect sizes between blood and BALF studies.

Although not disease-specific, KL-6 SP-D and IL-8 are useful biomarkers of SSc-ILD diagnosis, but verification of utility as a prognostic or severity indicator is still required. Similarly, while standardised effects of IL-8 appeared similar between blood and BALF estimates, the number of independent studies was limited, and further investigations should establish the best source of

biomarker measurement. In addition, we identified other mediators measured in both peripheral blood and BALF. CCL2, IL-10, HE4, HNP1 and MMP9 were increased in both compartments in SSc-ILD compared with healthy controls. TNF-alpha was increased in SSc-ILD BALF²⁹ and reduced when analysed in peripheral blood.³⁰ The differing constituents and concentrations suggest that SSc-ILD lung fibroblasts may be exposed to a different inflammatory milieu than other fibroblasts, which is an important parameter for in vitro models of fibroblasts reflecting SSc-ILD. There is a need for further investigations into the external validity of these mediators in order to support their utility as biomarkers in SSc-ILD management.

Combined biomarker panels, such as the secreted proteins measured in the peripheral blood as evidenced in this meta-analysis, could be useful for diagnostic management of SSc-ILD and effective prognostic practices^{11 50}; however, their combined value requires further research. A similar approach was recently proposed for progressively fibrosing ILD.⁵¹ Due to practical difficulties of repeated BALF, clinically and for patients, markers identified from both sources may have potential as diagnostic, prognostic or severity indicators, particularly when used together with markers for lung involvement and lung function.⁵² Such a mediator panel could improve our understanding of the in vivo inflammatory milieu which contributes to the development of fibrotic myofibroblasts in patients with SSc-ILD and could inform more accurate in vitro models for culture and subsequent investigation of the complex interaction of fibrotic myofibroblasts, pulmonary epithelial and immune cells in SSc-ILD. Repeated BAL procedures are not an ideal technique to monitor disease progression due to their invasive nature; however, they may support decision making where ILD needs further confirmation or characterisation. Distinctions and similarities in mediators between lung (BALF) and peripheral blood may help to evaluate organ-specific changes and in research settings.

Our protein-network and GO pathway analyses highlighted the involvement of TNF α , CCL2, IL-8 (CXCL8), IL10 and MMP9 in an inflammatory network while HNP1 (DEFA1) and HE4 (WFDC2) are not identified as part of such network. KL-6 (MUC1) and SP-D (SFTPD) also do not appear to be directly involved in the inflammatory network but are known indicators of severe alveolar barrier disruption when proteins 'leak' out of the airway epithelium during inflammation.^{52 53} Presenting soluble markers and their accompanying pathways suggested that the dominant peripheral blood and BALF mediators studied in SSc-ILD are mainly associated with a dysregulated immune response. Taken together, the identified secreted mediators are involved in various proinflammatory and profibrotic immune responses known to play a significant role in the pathophysiology of SSc-ILD, such as epithelial and fibroblast activation and ECM remodelling, as well as innate and adaptive immune activation.

Many of the identified soluble markers were only represented by a single study indicating a further need for extensive investigations into mediators in fibrotic lung diseases, which would support validation and inclusion in the quantitative synthesis.

We found a lack of universal terminology surrounding SSc-ILD. To prevent loss of information, all studies were included in this review regardless of whether patients received immunosuppressants, had undergone treatment or if information about lifestyle was available. The scope of this review was limited to soluble mediators and did not include cells, microRNAs or exhaled nitric oxide. Further, there was a lack of consistency in standardisation of lavage fluid procedures, with the number and volume of aliquots, and the percentage volume recovery varying

between studies.⁴⁷ Saline solution used for the bronchoalveolar lavage procedure dilutes the retrieved epithelial lining fluid, but the exact dilution factor and appropriate adjustment of concentrations were not possible and remain a challenge, particularly in evidence synthesis.^{24 54 55} Finally, different methodologies were used to determine KL-6 and IL-8 in the reported studies, which may limit comparability across studies, however, we used the SMD to minimise the impact on interpretation of effect size while also confirming minimal difference in subgroup estimates.

CONCLUSIONS

This systematic review of soluble mediators in blood and BALF from patients with SSc-ILD identified a large number of mediators differentially expressed compared with healthy controls illustrating the complexity of the interconnected signalling pathways that play a role in the pathogenesis of SSc-ILD. Further investigations present an opportunity for a greater understanding of the fibrotic mechanisms and potential therapeutic interventions in SSc-ILD. However, the value of individual biomarkers, as well as panel combinations, particularly for clinical assessment of SSc-ILD development and disease progression requires additional investigation and validation in large, longitudinal studies with well-characterised SSc-ILD cohorts.

Acknowledgements The authors thank AM Hoffmann-Vold and J Belperio (CX3CL1), and S O'Reilly (Gremlin-1) for sharing their original data.

Contributors Conception and design: IS and BCS. Data collection: AF, KNP, BA and BCS. Data analysis and interpretation: AF, KNP, RC, IS and BCS. Writing of the manuscript: AF, KNP, BA, IS and BCS. Final editing: IS and BCS. BCS is responsible for the overall content and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Funding This work was supported by the British Association for Lung Research (BALR) through a summer studentship to K Potel, (BALRSS21-04).

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data may be obtained from a third party and are not publicly available. All data relevant to the study are included in the article or uploaded as online supplemental information.

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Fields *et al.* Mediators in SSc-ILD

Mediators of systemic sclerosis-associated interstitial lung disease (SSc-ILD): systematic review and meta-analysis

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Supplementary Information

Supplement 1: Search strategy inputted into repositories

Our extensive searches performed on Embase, Web of Science, Medline ALL, Scopus, and PubMed consistently used the following format of search terms across all databases in string format: (“systemic sclerosis” AND “interstitial lung disease” AND “biomarker” AND (“plasma” OR “serum” OR “blood” OR “whole blood”)) OR (“systemic sclerosis” AND “interstitial lung disease” AND “biomarker” AND (“lung lavage” OR “bronchoalveolar lavage”)). The search was conducted on the 27th October 2021.

Supplement 2: Table of RoB assessment questions

Questions used with regards to domains of ‘Study Design and Methodology’, ‘Measurements and Results’ and ‘External Validity’ for assessment of RoB were based on CASP Checklist for Cohort Studies (https://casp-uk.b-cdn.net/wp-content/uploads/2018/03/CASP-Cohort-Study-Checklist-2018_fillable_form.pdf). Each question was individually scored (2 = appropriate, 1 = inappropriate or incomplete, 0 = uncertain) and weighted scores were calculated.

Study domain	Question	Weighing
Study design and methodology	<ol style="list-style-type: none"> 1. Did the study address a clearly focused issue? 2. Were the patient cohort and healthy controls recruited in an acceptable way? 3. Was SSc-ILD accurately diagnosed? 4. Were mediator levels accurately measured? 5. Were possible confounding factors identified and accounted for? 	x 2
Measurements and Results	<ol style="list-style-type: none"> 1. Were values accurately reported? 2. Were mediator levels accurately analysed? 3. Could the results be due to chance? 	x 3.3
External Validity	<ol style="list-style-type: none"> 1. Do the results of this study fit with other available evidence? 2. Can the results be applied to the local population? 	x 5

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Supplement 3: Table of genes and Ensembl IDs of statistically significant soluble markers identified in peripheral blood and BALF of patients with SSc-ILD used for g:Profiler analyses (1).

Marker	Gene	Ensembl ID
KL-6	MUC1	ENSG00000185499
SP-D	SFTPD	ENSG00000133661
TNFalpha	TNFA	ENSG00000232810
MMP-9	MMP9	ENSG00000100985
HNP1	DEFA1	ENSG00000206047
CCL2	CCL2	ENSG00000108691
HE4	WFDC2	ENSG00000101443
IL-10	IL10	ENSG00000136634
IL-8	CXCL8	ENSG00000169429

Fields *et al.* Mediators in SSc-ILD**Supplement 1:** Main study information extracted

Study	Study Type	Immuno-compromised excluded	Co-morbidities excluded	Outcome progression	Patients (n) / (ILD (n))	Female (%)	Age (yrs)	Controls (n)	Blood / BALF	Biomarker	Methodology
Andersen <i>et al</i> , 2007	Observational	no	ND [§]	no	9	7 (77.8%)	62.0 (13)	16	BALF	MMP-9, TIMP-1	ELISA
Benfante <i>et al</i> , 2018	Observational	yes	no	no	15	12 (80%)	44.2 ± 9.8	10	serum	SP-A, SP-D	ELISA
Bonella <i>et al</i> , 2011	Observational	no	yes	no	25	20 (80%)	55.52 ± 15.5	no information	serum	SP-D, KL-6	ELISA
d'Alessandro <i>et al</i> , 2021	Observational	ND [§]	ND [§]	yes	25	20 (80%)	62 (50-67)	23	serum	KL-6	Chemoluminescence Immunoassay
De Santis <i>et al</i> , 2011	Observational	no	ND [§]	yes	46	36 (78.2%)	55.1 ± 14	15	BALF	thymosin-β4	HPLC-ESI
Gao <i>et al</i> , 2020	Observational	ND [§]	ND [§]	no	exp: 102 val: 36 val: 105	77 (75.4%) 28 (77.8%) 89 (84.8%)	54.9 ± 11.9 48.4 ± 11.8 49.7 ± 12.6	10	serum	osteopontin, CCL18	ELISA
Gedik <i>et al</i> , 2020	Observational	yes	yes	no	42 / 20	40 (95.2%)	42 ± 11	38	serum	HNP1	ELISA
Grosicka <i>et al</i> , 2018	Observational	no	ND [§]	yes	41 / 29	41 (100%)	55 (29-60)	15	serum	SP-D	ELISA
Guiot <i>et al</i> , 2021	Observational	no	yes	yes	39	31 (79.5%)	61 ± 12	39	serum	total IGF-1, IGFBP-1, IGFBP-2, IGFBP-3, TGF-β1, YKL-40, CR, IL-8, TNF-α, MMP-7 and MMP-9	ELISA multiplex, ELISA
Habe <i>et al</i> , 2017	Observational	no	no	no	233 / 50	211 (90.5%)	65.4 ± 11.6	68	plasma	ADAMTS-13 activity, von Willebrand Factor, VWFpp, different fibrin-related markers	Immunological and enzyme activity methods
Hant <i>et al</i> , 2009	Observational	no*	no	no	44	36 (81.8%)	48 ± 11	10	serum	SP-D, KL-6	ELISA
Hasegawa <i>et al</i> , 2014	Observational	no	yes	yes	92 / 62	64 (69.6%)	53 (14-76)	24	serum	ICAM-1, E-selection, P-selectin	ELISA

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Hesselstrand <i>et al</i> , 2013	Observational	yes	ND ⁵	yes	15	9 (60%)	57 (49-68)	12	BALF, serum	CXCL5, CXCL8, S100A8/A9, COMP, ELISA, KL-6	Nanopia® KL-6 EIA
Hizal <i>et al</i> , 2015	Observational	no	ND ⁵	no	30	26 (86.6%)	64 ± 12.3	28	serum	LL-37	ELISA
Hoffmann-Vold <i>et al</i> , 2018	Observational	no	ND ⁵	yes	292	239 (81.8%)	48 ± 15.4	100	serum	CX3CL1	ELISA
Iannone <i>et al</i> , 2021	Observational	no	no	no	89	84 (94.3%)	52 ± 14	26	serum	adiponectin, leptin, resistin, visfatin, TNF-α, IFNγ, IL-2, IL-10, IL-17A	BioPlex 17A
Kennedy <i>et al</i> , 2015	Observational	no	ND ⁵	yes	6	6 (100%)	69.7 ± 8.4	10	serum	CCL18, TGF-β1, KL-6, SP-D, Thrombomodulin, MMP-7, PAI-1, and PDGF-AA, VCAM-1, ICAM-1, P-Selectin, L-Selectin, VEGF, TNF-α, and CCL2	Nanopia® KL-6 EIA, ELISA
Khadilkar <i>et al</i> , 2019	Observational	yes	yes	no	25 / 10	22 (88%)	35.4 ± 10.4	25	serum	IL-1β, IL-4, IL-6	no information
Kuzumi <i>et al</i> , 2021	Observational	yes	ND ⁵	yes	74 / 51	ILD: 36 (70.6%)	46.0 ± 16.2	14	serum	TARC	ELISA
Lee <i>et al</i> , 2012	Observational	no	yes	no	17	12 (68.4%)	51 (46-57)	27	plasma	Chitinase 1	Enzymatic assay
Mathai <i>et al</i> , 2010	Observational	yes	yes	no	12	9 (75%)	47.4 ± 3.1	27	plasma	CCL-18, IL10, M-CSF	ELISA
Meloni <i>et al</i> , 2004	Observational	ND ⁵	ND ⁵	no	28	22 (78.6%)	50.3 ± 8.9	9	BALF	IL-8, MCP-1, IL-12, IL-18, IL-10	no information
O'Reilly, 2021	Observational	yes	ND ⁵	no	21 / 5	18 (85.7%), ILD: 5 (100%)	47.9 ± 8.3; ILD: 54.6 ± 6.4	20	serum	Gremlin-1	ELISA
Sakamoto <i>et al</i> , 2015	Observational	yes	no	yes	33	23 (69.7%)	63 (54-70)	no information	BALF, plasma, serum	HNPs, IL-8	ELISA
Salazar <i>et al</i> , 2018	Observational	no	ND ⁵	yes	82	64 (78%)	53.6 ± 11.0	no information	plasma	KL-6, CCL-18	Nanopia® KL-6 EIA; ELISA
Sawicka <i>et al</i> , 2017	Observational	ND ⁵	yes	no	48 / 43	48 (100%)	34-84 (62 ± 10.6)	38	serum	resistin	ELISA

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Schmidt <i>et al</i> , 2009	Observational	no	ND [§]	yes	32 / 11	23 (71.9%)	58.5 (30–72)	15	BALF	IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, CCL2, CCL4, TNF- α , G-CSF, GM-CSF, and INF- γ	Bioplex, ELISA
Storkanova <i>et al</i> , 2021	Observational	no	ND [§]	yes	92 / 58	79 (85.9%)	55.0 (45.0–60.5)	92	plasma	Hsp90	ELISA
Takahashi <i>et al</i> , 2000	Observational	ND [§]	ND [§]	no	42 / 6	37 (88.1%) , ILD: 6 (100%)	54.9 \pm 9.3; ILD: 47.5 \pm 5.2	108	serum	SP-A, SP-D	ELISA
Taniguchi <i>et al</i> , 2018	Observational	yes	ND [§]	no	56	52 (92.9%)	59 (51.5–69)		serum	CXCL13	ELISA
Volkman <i>et al</i> , 2019	Interventional	ND [§]	ND [§]	yes	71 (CYC) 62 (MMF)	55 (77.5%) 44 (71.0%)	52.3 \pm 9.5 52.9 \pm 10.0	39	plasma	KL-6, CCL-18	Nanopia [®] KL-6 EIA, ELISA
Volkman <i>et al</i> , 2016	Interventional	ND [§]	ND [§]	yes	71 (CYC) 65 (MMF)	55 (77.5%) 45 (69.2%)	52.3 \pm 9.5 52.6 \pm 10.0	67	plasma	CXCL4	ELISA
Wakhlu <i>et al</i> , 2018	Observational	no	yes	no	93 / 55	90 (96.8%), ILD: 53 (96.4%)	37.8 \pm 10.3; ILD: 39 \pm 10	33	serum	IL-6, IL-17A, TGF β 1	ELISA
Yanaba <i>et al</i> , 2004	Observational	no*	ND [§]	yes	42 / 23	36 (85.7%)	9 \pm 18	30	serum	KL-6, SP-D	ELISA
Yanaba <i>et al</i> , 2013	Observational	no*	yes	no	71	58 (81.7%)	52 (17–77)	50	serum	VAP-1	ELISA
Zhang <i>et al</i> , 2020	Observational	no	yes	no	169 / 92	ILD: 62 (67.4%)	ILD: 61.3 \pm 19.7	169	BALF, serum	HE4	ELISA
Zheng <i>et al</i> , 2020	Observational	no	ND [§]	no	31 / 21	27 (87%), ILD: 17 (85%)	51 \pm 13; ILD: 53 \pm 12	20	serum	calpain, HMGB-1	Enzymatic activity, ELISA
Zolkiewicz <i>et al</i> , 2020	Observational	ND [§]	yes	no	22	21 (95.4%)	52.68 \pm 10.29	14	serum	PPAR- γ	ELISA

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Supplement 5:

Supplement 5a: Table of Markers in SSc-ILD identified in peripheral blood

↓ ↑	Marker	Unit	SSc-ILD		Control		Studies (n)	P value	Ref.
			Mean ± SD Median (*range,**IQR)	Samples (n)	Mean ± SD Median (*range,**IQR)	Samples (n)			
↓	Adams-TS (VWF _{FCP})	%	86.24 (46.34- 97.42) **	50	108 (55-155) ^{§**}	68	1	<0.0001	(2)
	IGF-1	ng/mL	9 (5.2-15.8)	39	13 (8-17)	39	1	<0.05	(3)
	IL-37	ng/mL	1.35 (0.16-1341.7)	30	5.53 (0.29-22659)	28	1	<0.05	(4)
	Selectin-L	pg/mL	745.7 (343.2-1423.9) *	62	1186 (379.3-2509.9) * [§]	24	1	<0.05	(5)
	TNF-α	pg/mL	3.40 ± 0.75	11	5.58 ± 1.47	27	1	<0.01	(6)
↑	Calpain	RFU/μL	0.5 ± 0.35 [§]	20	0.12 ± 0.16 [§]	20	1	<0.0001	(7)
	Chitinase 1	nM/mL/h	15.5 ± 2.4	17	8.1 ± 3.34	27	1	<0.0001	(8)
	CRP	mg/L	6.4 ± 9.1	39	1.2 ± 1.4	39	1	<0.001	(3)
	CCL17 (TARC)	pg/mL	636.1 ± 412.3	52	251.3 ± 89.4	14	1	<0.0001	(9)
	CCL18	ng/mL	152.6 (37.2-457.4) * 191.29 ± 11.08	82 133	84.6 (20.2-275.8) * 87.71 ± 28.28	40 39	2	<0.001 <0.001	(10) (11)
	CX3CL1 (Fractalkine)	ng/mL	2.5 ± 3.3	51	1.1 ± 1.4	100	1	<0.001	(12)
	CXCL13	ng/mL	95.5 (41.6-147.3) **	33	27.8 (22.2-35.8) **	16	1	<0.0001	(13)
	CXCL4 (Platelet factor 4)	ng/mL	2699 ± 1489	136	2233 ± 1351	67	1	<0.05	(14)
	Gremlin-1	ng/mL	20.69 ± 3.5	5	1.14 ± 0.7	20	1	<0.0001	(15)
	HMGB-1	ng/mL	12.85 ± 5.6 [§]	20	7.1 ± 3.4 [§]	20	1	<0.001	(7)
	Hsp90	ng/mL	13.9 (8.6-20.6) **	30	9.8 (7.7-12.4) **	92	1	<0.01	(16)
	ICAM-1	ng/mL	170.0 (89.7-474.8) *	62	108.7 (75.5-195.3) * [§]	24	1	<0.0001	(5)
	IGFBP-2	ng/mL	132 (85-213) **	39	83 (51-19)	39	1	<0.001	(3)
	IGFBP-3	ng/mL	656 (479-758) **	39	806 (675-926)	39	1	<0.05	(3)
	IL-1β	pg/mL	724.6 ± 534.0	10	5.318 ± 1.444	25	1	<0.01	(17)
	IL-1-RA	pg/mL	382.8 ± 165.4	11	111.2 ± 18.72	27	1	<0.05	(6)
	IL-4	pg/mL	21.25 ± 6.46	10	6.87 ± 2.94	25	1	<0.0001	(17)
	IL-6	pg/mL	4.49 ± 1.45 23.9 ± 12.4	10 55	1.83 ± 0.12 6.7 ± 3.9	25 38	2	<0.001 <0.0001	(17) (18)
	IL-17A	pg/mL	46.4 ± 14.8	55	16.4 ± 2.1	38	1	<0.0001	(18)
	KL-6 (MUC1)	U/mL	772.9 (116-4777) *	82	226.5 (97.9-712) *	40	8	<0.0001	(10)
			1752.05 ± 1274.67	133	330.7 ± 125.74	39		<0.0001	(11)
			1358.2 ± 952.5	15	202.5 ± 63	25		<0.0001	(19)
			1458 ± 1070	44	333 ± 294	10		<0.0001	(20)
			820 (400-1400) ** [§]	15	200 (160-240) ** [§]	12		<0.001	(21)
			473.7 ± 379.6 [§]	23	124.4 ± 89.6 [§]	30		<0.01	(22)
			1568 (1443-2065) **	13	239 (145-344) **	23		<0.0001	(23)
			836 (431- 1303) **	6	198 (52-360) **	10		<0.05	(24)
	Leptin	pg/mL	16155 ± 2564	51	2822 ± 2045	6	1	<0.0001	(25)
	MMP-7	ng/mL	5.41 (2.6- 7.2) **	6	0.0 (0.0-0.6) **	10	1	<0.001	(24)
	Osteopontin (OPN)	ng/mL	20.9 ± 10.9 [§]	102	9.11 ± 3.4 [§]	9	1	<0.001	(26)
	PPAR-γ	ng/mL	16.07 ± 2.4	11	13.92 ± 1.93	14	1	<0.05	(27)
	Resistin	ng/mL	10.5 ± 5.01	43	7.64 ± 4.43	38	1	<0.05	(28)
Selectin-E	ng/mL	36.1 (16.4-117) *	62	30.6 (14.3-80.2) * [§]	24	1	<0.05	(5)	
Selectin-P	ng/mL	745.7 (343.2-1423.9) *	62	90 (30.4-157.1) * [§]	24	1	<0.0001	(5)	
Sp-A	ng/mL	38.16 ± 17.04	30	26.7 ± 8.5	108	1	<0.01	(29)	
Sp-D	ng/mL	362 ± 285.2	15	22.9 ± 28.7	25	6	<0.0001	(19)	
		353 ± 219	44	40 ± 51	10		<0.0001	(20)	
		196 ± 64.9 [§]	23	47.3 ± 24.9 [§]	30		<0.00001	(22)	
		182.3 ± 118.9	30	46.4 ± 32.9	108		<0.0001	(29)	

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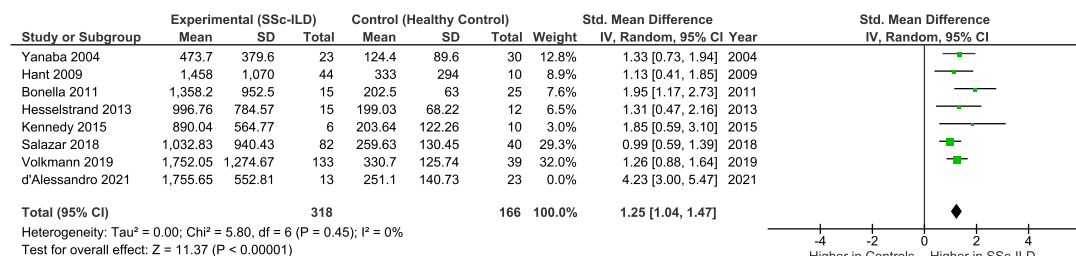
		420.1 ± 268.7	27	107 ± 34.4	27		<0.001	(30)
		115.3 ± 81.36	15	32 ± 11.9	10		<0.01	(31)
TGF-β1	pg/mL	1157 ± 296.6	55	377.2 ± 208.8	38	1	<0.0001	(18)
sVAP-1	ng/mL	520 (125-1520) * §	36	320.2 ± 144.2	50	1	<0.0001	(32)
VWFpp (Propeptide)	U/dL	117 (107-147) **	50	90 (40-155) ** §	51	1	<0.01	(2)
HNP1 # (Alpha defensin)	ng/mL	684 ± 473	20	377 ± 269	38	1	<0.05	(33)
CCL2 # (MCP-1)	pg/mL	557.7 ± 96.08	11	300.2 ± 43.25	27	1	<0.05	(6)
HE4 # (WFDC2)	pmol/L	172.1 (94.8-263.3) **	92	39.5 (24.3-54.2) **	169	1	<0.05	(34)
IL-10 #	pg/mL	18.94 ± 3.5	11	8.85 ± 1.16	27	1	<0.05	(6)
IL-8 #	pg/mL	11 (6-19) **	39	3.6 (1.5-7) **	92	3	<0.001	(3)
		8.7 (4.0-13.8) ** §	15	1.9 (1.3-2.6) ** §	12		<0.01	(21)
		16.4 (11.1-24.3) ** §	33	5.2 (3.9-11.6) ** §	20		<0.01	(35)
MMP-9 # (Gelatinase B)	ng/mL	1183 (482-1575) **	39	412 (221-818) **	39	1	<0.001	(3)

Present in peripheral blood and BALF.

§ Estimated values are taken from graphs where studies did not provide numerical values (<https://automeris.io/WebPlotDigitizer/>).

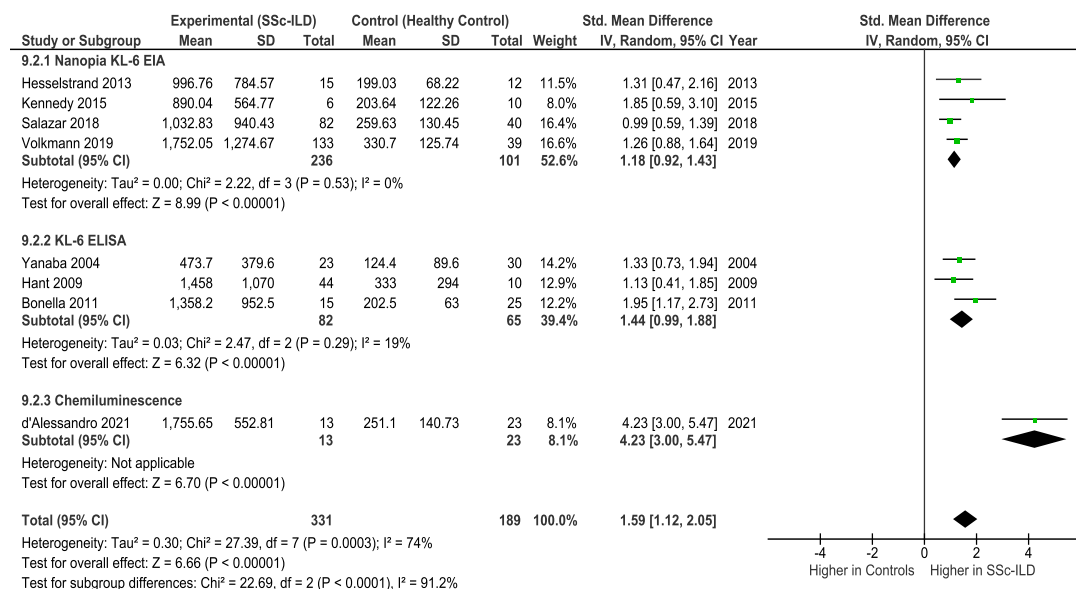
Supplement 5b: Sensitivity analyses for KL-6 in peripheral blood

Exclusion of the study by d'Alessandro *et al.* (23) reduced heterogeneity to moderate (I^2 : 26%), while not largely affecting the estimate due to the weighting of the excluded study.



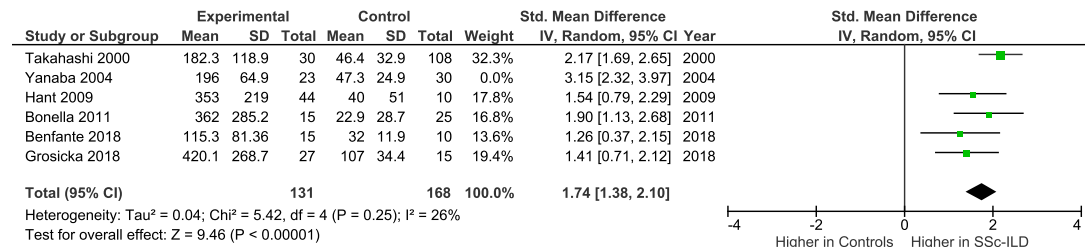
Fields *et al.* Mediators in SSc-ILD**Supplement 5c: Table of different methodologies used for analyses of KL-6, Sp-D and IL-8 in peripheral blood**

Analyte	Author	Technique used
Serum KL-6	Salazar GA, <i>et al.</i> 2018	Nanopia® KL-6 EIA
	Volkman ER, <i>et al.</i> 2019	Nanopia® KL-6 EIA
	Bonella F, <i>et al.</i> 2011	ELISA
	Hant FN, <i>et al.</i> 2009	ELISA
	Hesselstrand R, <i>et al.</i> 2013	Nanopia® KL-6 EIA
	Yanaba K, <i>et al.</i> 2004	ELISA
	D'Alessandro M, <i>et al.</i> 2021	Chemoluminescence Enzyme Immunoassay
	Kennedy B, <i>et al.</i> 2015	Nanopia® KL-6 EIA
Serum Sp-D	Bonella F, <i>et al.</i> 2011	ELISA
	Hant FN, <i>et al.</i> 2009	ELISA
	Yanabe K, <i>et al.</i> 2004	ELISA
	Takahashi H, <i>et al.</i> 2000	ELISA
	Grosicka A, <i>et al.</i> 2018	ELISA
	Benfante A, <i>et al.</i> 2018	ELISA
Serum IL-8	Guiot J, <i>et al.</i> 2021	Fluorokine-1 Multianalyte Profiling ELISA
	Hesselstrand R, <i>et al.</i> 2013	ELISA
	Sakamoto N, <i>et al.</i> 2015	ELISA

Supplement 5d: Sub analyses by methodology (peripheral blood KL-6)

Fields *et al.* Mediators in SSC-ILD**Supplement 5e: Sensitivity analyses for Sp-D in peripheral blood**

Exclusion of the study by Yanaba *et al.* (22) reduced heterogeneity to moderate (I^2 : 26%), while not largely affecting the estimate due to the weighting of the excluded study.



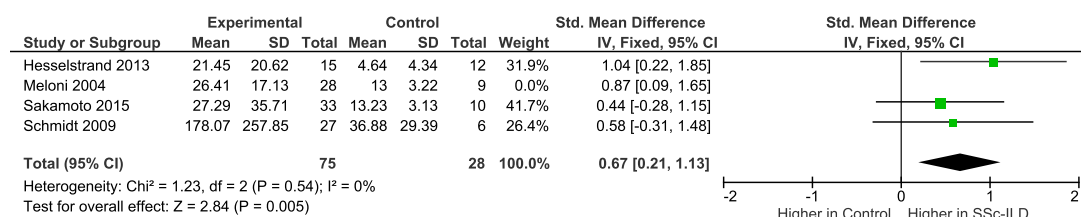
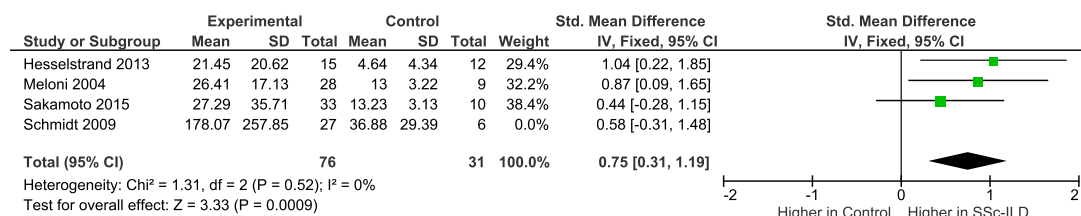
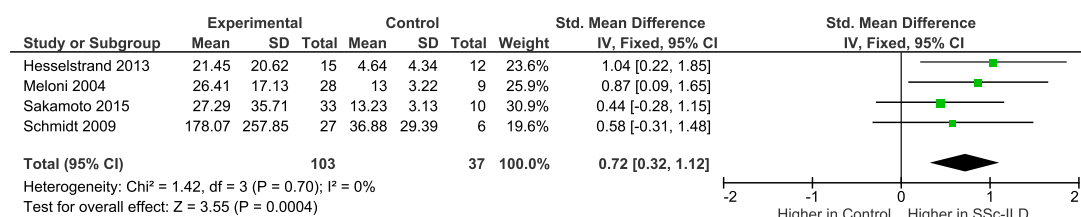
Fields *et al.* Mediators in SSc-ILD**Supplement 6: Table of pulmonary markers in SSc-ILD found in BALF**

	Marker	Unit	SSc-ILD		Control		Studies (n)	P value	Ref.
			Mean ± SD Median (*range, **IQR) †	Samples (n)	Mean ± SD Median (*range, **IQR) †	Samples (n)			
↑	CXCL5 (ENA-78)	ng/mL	12.1 (2.3-28.3) ** †	15	1.1 (0.6-2.2) ** †	12	1	<0.01	(21)
	CCL4 (MIP-1β)	pg/mL	46.0 (24.6-350) *	27	21.6 (2.8-58.8) *	6	1	<0.01	(36)
	IL-12	pg/mL	11.79 (2.38-24.69) ** †	28	0.36 (0.33-0.44) ** †	9	1	<0.01	(37)
	IL-7	pg/mL	4.88 (0.75-17.4) *	27	2 (0-8.2) *	6	1	<0.05	(36)
	Pro-MMP-9 (Pro-Gelatinase B)	ng/mL	3.0 ± 3.7	9	1.2 ± 1.3	16	1	<0.05	(38)
	S100A8/09 (Calprotectin, MRP8/MRP14)	μg/mL	1.97 (0.58-3.78) ** †	15	0.05 (0.01-1.1) ** †	12	1	<0.05	(21)
	TIMP-1	ng/mL	14.3 (3.7-24.9) **	16	7 (5.7-8.7) **	16	1	<0.05	(38)
	TNF-α #	pg/mL	1.2 (0-8.1) *	27	0 (0-0.6) *	6	1	<0.01	(36)
	Tβ4 (Thymosin beta-4)	μmol/L	0.31 ± 0.37	46	0.11 ± 0.08	15	1	<0.05	(39)
	HNP 1-3 # (α-defensin)	pg/mL	240.0 (26.4-563.1) **	33	79.7 (65.6-107.8) **	20	1	<0.01	(35)
	CCL2 # (MCP-1)	pg/mL	92.2 (14.1-2001) *	27	31.6 (14.4-42.6) *	6	1	<0.01	(36)
	HE4 # (WFDC2)	pmol/L	754.4 (299-1060) **	92	238.7 (97.7-397.6)	37	1	<0.001	(34)
	IL-10 #	pg/mL	1.5 (0-6.25) ** †	28	0 (0) ** †	9	1	<0.01	(37)
	IL-8 #	pg/mL	15.6 (5.5-32.4) ** † 15.4 (9.7-33.1) ** † 24.5 (6.5-46.6) ** 106 (14.9-794) *	15 33 28 27	3.4 (1.7-6.0) ** † 14.4 (2.9-17.3) ** † 13.7 (8.8-16.5) ** 31 (0.1-112.2) *	12 10 9 6	4	<0.001 <0.01 <0.05 <0.05	(21) (35) (37) (36)
	MMP-9 # (Gelatinase B)	ng/mL	0.4 (0.3-0.5) **	9	0.2 (0.01-0.4) **	16	1	<0.01	(38)

Present in peripheral blood and BALF. 40 (30-57.5)

† Estimated values are taken from graphs where studies did not provide numerical values (<https://automeris.io/WebPlotDigitizer/>).**Supplement 6b: Table of different methodologies used for the analyses of IL-8 in BALF**

Analyte	Author	Technique used
BALF IL-8	Hesselstrand R, <i>et al.</i> 2013	ELISA
	Sakamoto N, <i>et al.</i> 2015	ELISA
	Meloni F, <i>et al.</i> 2004	No information
	Schmidt K, <i>et al.</i> 2009	Bioplex

Fields *et al.* Mediators in SSc-ILD**Supplement 6c: Sensitivity analyses for IL-8 in BALF**Sensitivity analysis with exclusion of the study by Schmidt *et al.* (36) or Meloni *et al.* (37)However, exclusion of this study did not change the heterogeneity (I^2 : 26%) or the direction of the estimate.

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Supplement 7:

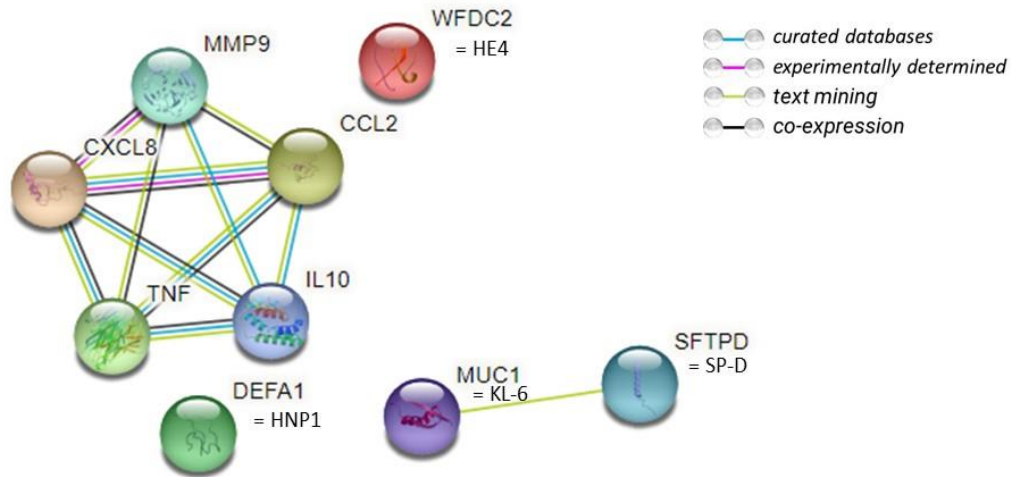


Figure S7: String analyses of protein-protein association network of the identified secretome identified in SSc-ILD. Network nodes represent the differentially secreted proteins identified in this review. Edges (lines) represent protein-protein associations that are specific and meaningful, such as proteins that jointly contribute to a shared function (note that this does not necessarily mean they physically bind each other). Thickness of network edges indicates the strength of data support. The minimum required interaction score was set to a high confidence level of 0.7 for our data analysis.

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