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Research article

# High Interleukin-13 level is associated with disease stability in interstitial Lung disease

Elena K. Joerns [a,b,*], David Karp [a], Song Zhang [c], Jeffrey A. Sparks [d], Traci N. Adams [e], Una E. Makris [a,f], Chad A. Newton [e]

[a] University of Texas Southwestern Medical Center, Department of Internal Medicine, Division of Rheumatic Diseases, Dallas, TX, USA
[b] Mayo Clinic, Department of Internal Medicine, Division of Rheumatology, Rochester, MN, USA
[c] University of Texas Southwestern Medical Center, Department of Population and Data Sciences, Division of Biostatistics, Dallas, TX, USA
[d] Brigham and Women's Hospital, Department of Medicine, Division of Rheumatology, Inflammation and Immunity, Harvard Medical School, Boston, MA, USA
[e] University of Texas Southwestern Medical Center, Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, Dallas, TX, USA
[f] Dallas Veterans Affairs Medical Center, Dallas, TX, USA

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ABSTRACT (144/150 WORDS)

*Purpose:* Cytokines can help predict prognosis in interstitial lung disease (ILD) and to differentiate between ILD subtypes. The objectives of our study were to evaluate association of baseline cytokine levels with time to ILD progression and to compare baseline cytokine levels between ILD subtypes.
*Methods:* We quantified 27 cytokines using a multiplex assay in peripheral blood samples from 77 patients. Cox proportional hazards regression analysis was performed to evaluate cytokine impact on the time to progression in the total cohort and within each ILD type. We evaluated for significant differences in cytokine levels between ILD types using ANOVA, Wilcoxon signed-rank test and Tukey method.
*Results:* Higher IL-13 level was associated with longer time to progression (hazard ratio 0.52 [0.33–0.81], p-value 0.004). FGF-β, GM-CSF, and IL-17 levels differed significantly between fibrotic and inflammatory ILD subgroups.
*Conclusion:* IL-13 may be a useful biomarker predicting ILD stability.

## 1. Introduction

Interstitial lung disease (ILD) involves progressive lung inflammation and fibrosis which contributes significantly to morbidity and mortality. Multiple subtypes of ILD exist; the classification hinges on etiology and relies on careful history taking, physical examination, chest radiography, serologies, as well as biopsy, if needed [1]. Idiopathic pulmonary fibrosis (IPF) is a disease with unclear pathogenesis that exhibits relentless progressive fibrosis and very poor prognosis that is generally treated with antifibrotics [2]. Other subtypes such as chronic hypersensitivity pneumonitis (cHP) and rheumatic disease (RD)-associated ILD likely have an inflammatory etiology due to an antigen exposure in cHP and autoimmune disease in RD-ILD; both are often treated with immunosuppression [3]. Unclassifiable ILD (uILD) presents a unique challenge in management due to conflicting findings and lack of etiology-based ILD

* Corresponding author.
*E-mail address:* joerns.elena@mayo.edu (E.K. Joerns).

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categorization [4]. Interstitial pneumonia with autoimmune features (IPAF) represents an important subcategory of unclassifiable ILD. IPAF was defined in 2015 to classify a subgroup of patients for research purposes who appear to have an inflammatory etiology to their ILD and exhibit autoimmune features such as positive serologies or inflammatory pattern of lung lesion, but who do not have a clearly defined RD [5].

In multiple types of ILD, progressive fibrosis leads to lung function loss, decreased quality of life, oxygen requirements, and early death [3]. Mechanisms of disease progression in ILD have been proposed including increased inflammation [6,7], epithelial damage [8], telomere shortening [9], and genotype-environment interactions [10]. It is, however, unclear whether each type of ILD progresses via a separate mechanism or if progressive fibrosing ILD (PF-ILD) is a unique phenotype with one distinct pathomechanism. Markers associated with progression in IPF such as interleukin [IL]-6 and C-X-C ligand motif 13 (CXCL-13) were found to be relevant in other ILD types [11,12], suggesting that a unique phenotype associated with progression may be conserved in ILD, regardless of type. A definition for progressive pulmonary fibrosis (PPF) was recently proposed to include two of the following: annual forced vital capacity (FVC) decline of $\geq$5 % or diffusing capacity of lung for carbon monoxide (DLCO) decline of $\geq$10 %, increase in fibrosis severity on chest imaging, and/or increase in respiratory symptoms with no alternative explanation [13]. Prior definitions have included annual FVC decline of $\geq$10 %, DLCO decline of $\geq$15 %, and/or increase in fibrosis severity, usually accompanied by an increase in symptoms [14]. However, this definition only addresses the phenotype after the irreversible lung function loss has already occurred. There is a sparcity of biomarkers that are clinically used to predict outcomes and PPF behavior in patients with ILD. Therefore, a search for biomarkers for identification of the PPF phenotype prior to ILD progression is of great need.

There has been great interest in identifying biomarkers for differentiation of unique subtypes within ILDs, a goal especially relevant in unclassifiable types including IPAF, where identifying exact etiology may not always be possible. Multiple blood based biomarkers, such as Krebs von den Lungen-6 (KL-6), surfactant protein A (SP-A), and surfactant protein D (SP-D), suggested ongoing lung damage in studies [15,16]. KL-6 may be a prognostic marker of epithelial damage in ILD [16]. Additionally, transforming growth factor-β (TGF-β) was identified as a molecular driver in IPF progression due to its role in fibroblast activity [17]. But, only few of the studied biomarkers have been found to have discriminatory capacity between ILD subtypes. Some markers such as C-X-C motif ligand 9 (CXCL9), CXCL10, and CXCL11 associate with autoimmunity in ILD [18]. A combination of osteopontin, matrix metalloproteinase 7 (MMP7), and SP-D identified IPF as opposed to non-IPF ILD [19]. Cytokines act as cellular mediators, reflecting the underlying inflammatory processes and immune mechanisms [20]. Various cytokines such as IL-4, IL-6, and IL-13, have been implicated in pathogenesis of different forms of lung fibrosis, without necessarily differentiating between the ILD types [21]. There remains an urgent need to identify biomarkers which would reliably differentiate between ILD subtypes and allow for precise diagnosis and accurate management as well as help avoid invasive procedures [22].

Based on prior studies, we hypothesized that cytokines may be useful biomarkers for both identifying disease activity and for differentiating between ILD subtypes due to their correlation to various disease mechanisms in ILD [23]. The objectives of our study were to 1) evaluate association of cytokine levels with ILD progression, 2) establish unique cytokine profiles by comparing baseline cytokine levels between ILD subtypes, and 3) evaluate association of baseline cytokine levels with time to progression within each ILD subtype.

## 2. Methods

### 2.1. Study design, participant, and classification of ILD

We conducted a retrospective observational study to evaluate associations of baseline cytokine levels with progression-free survival and with the ILD subtype classification. We included patients seen in University of Texas Southwestern (UTSW) ILD clinic between June 2017 and March 2020. Patients were included if they had a multidisciplinary diagnosis of cHP, RD-ILD, IPAF, IPF, or uILD, and who were treatment naïve at the time of blood collection. Patients were separated into ILD classification groups based on American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) criteria for RD-ILD [24–28], American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria for IPAF [5], and ATS/ERS criteria for IPF [2]. CHP and uILD patients were classified according to multidisciplinary discussion among UTSW pulmonary, radiology, and pathology providers. A rheumatologist (EKJ) additionally performed medical record review to verify classifications of RD-ILD, IPAF, and uILD patients. Classifications were discussed with pulmonologist (CAN) and consensus was reached. Patients were excluded if they were on immunosuppressive or antifibrotic therapy at the time of blood collection to minimize confounding by treatment. This study was approved by UTSW institutional review board (protocol #092017-007). All patients provided written informed consent as part of research blood draw for participation in this study.

### 2.2. Sample processing and cytokine biomarker measurement

Serum was isolated from peripheral blood then aliquoted and stored at −80 °C, until sample analysis. Multiplex measurement was performed to quantify 27 cytokines (eotaxin, fibroblast growth factor basic [FGF-β], granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon gamma [IFN-γ], IL-1β, IL-1-receptor antagonist [IL-1-RA], IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ-induced protein 10 [IP-10], monocyte chemoattractant protein 1 [MCP-1], macrophage inflammatory protein [MIP]-1α, MIP-1β, platelet derived growth factor [PDGF]-BB, regulated upon activation, normal T cell expressed and presumably secreted [RANTES], tumor necrosis factor [TNF]-α, and vascular endothelial growth factor [VEGF]) according to manufacturer's instructions (Supplementary Appendix A). Cytokine assay was chosen to include

those associated with pathogenesis of autoimmune and fibrotic diseases.

### 2.3. Survival analysis

The primary endpoint was time from blood sample collection to progression. Progression was defined as a composite of relative FVC % decline of ≥10 %, death due to any cause, or lung transplant. Due to the retrospective nature of the data collection, we did not utilize the 2022 PPF definition [6] due to difficulty ascertaining respiratory symptoms and high potential for missing imaging and clinical data. Entry point into the cohort was the date of the research blood draw (June 2017 to March 2020). Baseline FVC was considered the first FVC from the date of the blood draw ( ± 3 months). We calculated time to progression after the baseline PFTs.

### 2.4. Statistical analysis

Cytokine levels below the detectable threshold were set to 0. Then we added one to each of the cytokine values and natural log-transformed them due to skewed distribution. Descriptive statistics were used to describe the cohort with categorical variables being expressed as counts with percentages and continuous variables as mean with standard deviation. We compared baseline characteristics of the ILD subtype groups using Fisher's exact and Kruskall-Wallis tests, as appropriate. All these tests were 2-sided with p-value of $<0.05$ considered significant.

We utilized Cox proportional hazards regression analysis to evaluate for the association between cytokines and time to progression with Breslow method for ties handling. Cytokines were handled as continuous (using maximum likelihood estimate) and categorical variables (separated into quartiles) in separate analyses. We assessed the significance of our findings in the cytokine analysis by controlling the 10 % false discovery rate (FDR) using Benjamini-Hochberg procedure [29].

We analyzed associations of cytokines with time to progression while controlling for potential confounders. Confounders were chosen based on biologic plausibility and significance in univariate analysis ($p < 0.05$). They included Gender-Age-Physiology (GAP) Index. GAP was included as a covariate in the Cox regression models. Cytokines significantly associated with survival, were separated into quartiles based on serum level and Kaplan-Meier curves were constructed to visualize patient survival. We separately performed

**Table 1a**
Baseline characteristics of the cohort (n = 77) separated by ILD diagnosis.

| | Total cohort (n = 77) | cHP (n = 12) | IPAF (n = 9) | IPF (n = 33) | RD-ILD (n = 12) | uILD (n = 11) | p |
|---|---|---|---|---|---|---|---|
| Female sex, n (%) | 27 (35.1) | 4 (33.3) | 6 (66.7) | 6 (18.2) | 8 (66.7) | 3 (27.3) | <0.0001 |
| Age at initial visit in years, mean (SD) | 71.60 (9.48) | 72.58 (7.76) | 73.69 (7.65) | 74.16 (7.39) | 63.78 (14.09) | 69.70 (8.51) | 0.14 |
| GAP Index, mean (SD) | 4.05 (1.56) | 4.67 (1.56) | 3.78 (1.30) | 4.18 (1.45) | 3.08 (1.44) | 4.27 (1.85) | 0.11 |
| Hispanic ethnicity, n (%) | 7 (9.1) | 1 (8.3) | 1 (11.1) | 2 (6.1) | 2 (16.7) | 1 (9.1) | 0.017 |
| Race, n (%) | | | | | | | 0.023 |
| Black | 1 (1.3) | 1 (8.3) | 0 | 0 | 0 | 0 | |
| Asian | 1 (1.3) | 0 | 0 | 0 | 0 | 1 (9.1) | |
| White | 75 (97.4) | 11 (91.7) | 9 (100.0) | 33 (100.0) | 12 (100.0) | 10 (90.9) | |
| Never smoker, n (%) | 28 (36.4) | 2 (16.7) | 2 (22.2) | 14 (42.4) | 7 (58.3) | 3 (27.3) | 0.0003 |
| ILD classification, n (%) | | | | | | | |
| CHP | 12 (15.6) | | | | | | |
| IPAF | 9 (11.7) | | | | | | |
| IPF | 33 (42.9) | | | | | | |
| RD-ILD | 12 (55.6) | | | | | | |
| UILD | 11 (14.3) | | | | | | |
| UIP pattern on imaging, n (%) | | | | | | | <0.0001 |
| Definite UIP | 18 (23.4) | 5 (41.7) | 0 | 10 (30.3) | 3 (25.0) | 0 | |
| Possible UIP | 33 (42.9) | 2 (16.7) | 2 (22.2) | 23 (69.7) | 4 (33.3) | 2 (18.2) | |
| Inconsistent with UIP | 26 (33.8) | 5 (41.7) | 7 (77.8) | 0 | 5 (41.7) | 9 (81.8) | |
| Years of follow up, mean (SD) | 1.81 (1.22) | 1.46 (1.22) | 1.54 (1.00) | 2.25 (1.29) | 1.66 (1.09) | 1.27 (1.06) | 0.12 |
| Initial %FVC, mean (SD) *missing: 1* | 71.00 (19.44) | 75.25 (14.64) | 71.00 (18.49) | 73.54 (20.50) | 68.18 (24.07) *missing: 1* | 61.54 (15.82) | 0.42 |
| Initial %DLCO, mean (SD) *missing: 3* | 50.32 (19.25) | 54.08 (14.00) | 53.67 (12.01) | 52.52 (23.69) *missing: 2* | 45.00 (17.65) *missing: 1* | 42.64 (15.53) | 0.38 |
| Familial pulmonary fibrosis, n (%) | 24 (31.2) | 4 (33.3) | 4 (44.4) | 10 (30.3) | 3 (25.0) | 3 (27.3) | 0.0038 |
| Duration of disease prior to blood draw in years, mean (SD) | 2.44 (2.58) | 2.17 (1.66) | 2.18 (2.80) | 2.48 (2.63) | 2.79 (3.56) | 2.42 (2.22) | 0.99 |
| Outcomes, n (%) | | | | | | | 0.0002 |
| Lung Transplant | 4 (9.1) | 1 (14.3) | 0 | 2 (10.0) | 0 | 1 (20.0) | |
| Relative FVC decline of ≥10 % | 31 (70.5) | 4 (57.1) | 3 (50.0) | 14 (70.0) | 6 (100.0) | 4 (80.0) | |
| Death | 9 (20.4) | 2 (28.6) | 3 (50.0) | 4 (20.0) | 0 | 0 | |

SD – standard deviation; GAP – Gender, Age, Physiology; ILD – interstitial lung disease; cHP – chronic hypersensitivity pneumonitis; IPAF – interstitial pneumonia with autoimmune features; IPF – idiopathic pulmonary fibrosis; RD-ILD – rheumatic disease-associated interstitial lung disease; uILD – unclassifiable interstitial lung disease; UIP – usual interstitial pneumonia; FVC – forced vital capacity; DLCO – diffusing capacity of lung for carbon monoxide.

multivariable survival analysis to identify significant variables associated with time to progression using stepwise forward logistic regression where we included all baseline variables and cytokines.

Secondary outcome included cytokine differences based on ILD classification. We evaluated for significant differences in cytokine levels between ILD subtypes using ANOVA, Wilcoxon signed-rank test, and Tukey comparison. Cytokines were only considered to be significantly different between groups when all three tests demonstrated difference with $p < 0.05$. Due to small number of patients within each ILD subtype, we combined cHP and RD-ILD into inflammatory ILD subtype and IPAF and uILD into unclassifiable ILD subtype. IPF represented fibrotic ILD subtype. We constructed violin plots to visualize significant differences in cytokines between ILD subtypes.

Within each ILD subtype, we performed Cox regression analysis to evaluate for significant associations between cytokine levels and time to progression. Each analysis was adjusted for GAP. Analyses were completed in Stata (software package Stata V.17, College Station, TX) and SAS 9.4 (SAS Institute, Cary, NC).

## 3. Results

### 3.1. Baseline characteristics

We identified 77 patients with ILD not receiving any treatment (antifibrotic or immunosuppressant) at baseline among the 234 patients in our cohort.

The subjects were predominantly male, White, Non-Hispanic population, with 64 % former smokers (Tables 1a and 1b). Twelve patients had cHP, nine patients had IPAF, 33 had IPF, 12 had RD-ILD, and 11 had uILD. There were more women within RD-ILD and IPAF groups than in other ILD categories. A radiographic UIP pattern was more common among IPF patients as compared to other subtypes. More RD-ILD patients had mild fibrosis as compared to other subtypes who had moderate or severe fibrosis more frequently. Additionally, high proportion of IPAF patients had familial pulmonary fibrosis. None of the other baseline characteristics differed significantly between ILD subtypes. When the groups were combined into inflammatory, unclassifiable, and fibrotic types, the distribution of female sex, UIP and years of follow-up differed significantly between the groups (Table 1b). The UIP distribution remained similar after combining into unclassifiable and inflammatory groups. However, the female sex was more predominant in RD-ILD and IPAF groups than in cHP and uILD groups, decreasing the female sex percentage after combination into broader groups.

We evaluated for baseline characteristics associated with progression (Table 2). Hispanic ethnicity was associated with progression (HR 2.61, 95 % CI 1.66–3.56, p 0.047) despite comprising only nine percent of the total cohort. GAP was significantly associated with

**Table 1b**
Baseline characteristics of the cohort (n = 77) separated by ILD type.

| | Total cohort (n = 77) | Inflammatory (n = 24) | Unclassifiable (n = 20) | Fibrotic (n = 33) | p |
|---|---|---|---|---|---|
| Female sex, n (%) | 27 (35.1) | 12 (50.0) | 9 (45.0) | 6 (18.2) | 0.025 |
| Age at initial visit in years, mean (SD) | 71.60 (9.48) | 68.18 (12.00) | 71.50 (8.18) | 74.16 (7.39) | 0.24 |
| GAP Index, mean (SD) | 4.05 (1.56) | 3.88 (1.68) | 4.05 (1.60) | 4.18 (1.45) | 0.79 |
| Hispanic ethnicity, n (%) | 7 (9.1) | 3 (12.5) | 2 (10.0) | 2 (6.1) | 0.70 |
| Race, n (%) | | | | | 0.23 |
| Black | 1 (1.3) | 1 (4.2) | 0 (0) | 0 | |
| Asian | 1 (1.3) | 0 (0) | 1 (5.0) | 0 | |
| White | 75 (97.4) | 23 (95.8) | 19 (95.0) | 33 (100.0) | |
| Never smoker, n (%) | 28 (36.4) | 9 (37.5) | 5 (25.0) | 14 (42.4) | 0.45 |
| ILD classification, n (%) | | | | | <0.0001 |
| CHP | 12 (15.6) | 12 (50.0) | 0 (0) | 0 (0) | |
| IPAF | 9 (11.7) | 0 (0) | 9 (45.0) | 0 (0) | |
| IPF | 33 (42.9) | 0 (0) | 0 (0) | 33 (100) | |
| RD-ILD | 12 (55.6) | 12 (50.0) | 0 (0) | 0 (0) | |
| UILD | 11 (14.3) | 0 (0) | 11 (55.0) | 0 (0) | |
| UIP pattern on imaging, n (%) | | | | | <0.0001 |
| Definite UIP | 18 (23.4) | 8 (33.3) | 0 (0) | 10 (30.3) | |
| Possible UIP | 33 (42.9) | 6 (25.0) | 4 (20.0) | 23 (69.7) | |
| Inconsistent with UIP | 26 (33.8) | 10 (41.7) | 16 (80.0) | 0 | |
| Years of follow up, mean (SD) | 1.81 (1.22) | 1.56 (1.14) | 1.39 (1.01) | 2.25 (1.29) | 0.028 |
| Initial %FVC, mean (SD) *missing: 1* | 71.00 (19.44) | 71.87 (19.59) *missing: 1* | 65.80 (17.29) | 73.54 (20.50) | 0.42 |
| Initial %DLCO, mean (SD) *missing: 3* | 50.32 (19.25) | 49.74 (16.16) *missing: 1* | 47.60 (14.81) | 52.52 (23.69) *missing: 2* | 0.38 |
| Familial pulmonary fibrosis, n (%) | 24 (31.2) | 5 (20.8) | 9 (45.0) | 10 (30.3) | 0.25 |
| Duration of disease prior to blood draw in years, mean (SD) | 2.44 (2.58) | 2.48 (2.74) | 2.31 (2.43) | 2.48 (2.63) | 0.97 |
| Outcomes, n (%) | | | | | 0.96 |
| Lung Transplant | 4 (9.1) | 1 (7.7) | 1 (9.1) | 2 (10.0) | |
| Relative FVC decline of ≥10 % | 31 (70.5) | 10 (76.9) | 7 (63.6) | 14 (70.0) | |
| Death | 9 (20.4) | 2 (15.4) | 3 (27.3) | 4 (20.0) | |

progression (HR 1.46, 95 % CI 1.16–1.83, p 0.001).

### 3.2. Primary outcome: cytokines associated with progression

In unadjusted Cox regression, higher IL-13 levels were associated with lower hazard ratio of progression (HR 0.52, 95 % CI 0.33–0.81, p 0.004, significant at 10 % FDR). High IP-10, MIP-1β, and RANTES levels were associated with faster onset of progression in unadjusted analysis, although this was not significant when adjusted for multiple comparisons at FDR 10 % (Table 3). When adjusted for GAP, the association was significant for IL-13 and RANTES, but not for IP-10 or MIP-1β. However, the association of neither IL-13 nor RANTES was significant when corrected for FDR 10 % in adjusted analysis (Table 3). Notably, due to high proportion of values being below detectable level for IFN-γ, IL-5, and IL-15 these cytokines were not analyzed for time to progression association.

When separated into quartiles, highest IL-13 quartile was associated with lowest hazard of progression (HR 0.235, 95 % CI 0.094–0.59, p 0.0065) (Fig. 1).

In a stepwise forward Cox proportional hazard regression including all cytokines and baseline variables, IL-13 and IL-1β were the only two biomarkers associated with time to progression (Table 4). Higher GAP and having IPAF classification were also associated with higher risk for faster progression in this analysis.

### 3.3. Secondary outcome: differences in cytokine levels by ILD subtypes

No cytokines differed significantly between individual ILD types with the exception of GM-CSF, which differed significantly between IPF and cHP (p-value 0.040) (data not shown).

FGF-β, GM-CSF, and IL-17 levels were significantly higher in inflammatory ILD (cHP and RD-ILD patients) than in IPF (Fig. 2, Table 5). Eotaxin level differed between inflammatory and unclassifiable (IPAF and uILD) ILD subgroups (Table 5).

### 3.4. Analysis of cytokine level association with time to progression within each ILD subtype

As an exploratory analysis, we analyzed association of individual cytokines with time to progression within each combined ILD subgroup (inflammatory, fibrotic, and unclassifiable).

Within the inflammatory group, we found that IL-1-RA was associated with faster progression (HR 2.78, 95%CI 1.06–7.29, p = 0.038). Higher IL-13 level was associated with longer time to progression in the inflammatory group, but it was not statistically significant (HR 0.68, 95%CI 0.27–1.68, p = 0.398). None of the cytokines were significantly associated with progression when adjusted for GAP (Appendix Table 6).

Within the unclassifiable group, we found that there was a trend for higher IL-13 level association with longer time to progression (HR 0.47, 95%CI 0.21–1.05, p = 0.067). Increasing IP-10 level was significantly associated with faster progression (HR 4.86, 95%CI

**Table 2**
Baseline characteristics associated with time to progression (n = 77).

| | HR | 95 % CI | p |
|---|---|---|---|
| Female sex | 1 | 0.34-1.70 | 0.95 |
| Hispanic ethnicity | 2.61 | 1.66-3.56 | 0.047 |
| Race | | | |
| White | Referent | | |
| Black | 12.44 | 10.32-14.57 | 0.020 |
| Asian | 6.46 | 4.40-8.52 | 0.075 |
| Never smoker | 0.74 | 0.39-1.40 | 0.35 |
| ILD Classification | | | |
| CHP | Referent | | |
| IPAF | 1.28 | 0.43-3.87 | 0.66 |
| IPF | 0.64 | 0.27-1.53 | 0.31 |
| RD-ILD | 0.86 | 0.29-2.60 | 0.79 |
| UILD | 0.95 | 0.30-3.01 | 0.93 |
| UIP Pattern | | | |
| Definite UIP | Referent | | |
| Possible UIP | 0.56 | 0.27-1.15 | 0.12 |
| Inconsistent with UIP | 0.70 | 0.32-1.55 | 0.38 |
| Familial pulmonary fibrosis | 1.23 | 0.66-2.27 | 0.52 |
| Age at initial visit | 0.98 | 0.95-1.01 | 0.22 |
| GAP Index | 1.46 | 1.16-1.83 | 0.001 |
| Initial FVC | 0.98 | 0.97-1.00 | 0.067 |
| Initial DLCO | 1.00 | 0.98-1.01 | 0.75 |
| Duration of disease prior to blood draw | 0.96 | 0.84-1.10 | 0.57 |

HR – hazard ratio; CI – confidence interval; ILD – interstitial lung disease; cHP – chronic hypersensitivity pneumonitis; IPAF – interstitial pneumonia with autoimmune features; IPF – idiopathic pulmonary fibrosis; RD-ILD – rheumatic disease-associated interstitial lung disease; uILD – unclassifiable interstitial lung disease; UIP – usual interstitial pneumonia; GAP – Gender, Age, Physiology; FVC – forced vital capacity; DLCO – diffusing capacity of lung for carbon monoxide.

**Table 3**
Individual cytokine analysis for association with ILD progression (n = 77).

| Cytokine | Unadjusted | | | Adjusted for GAP Index | | |
|---|---|---|---|---|---|---|
| | HR | 95 % CI | p | HR | 95 % CI | p |
| Eotaxin | 0.53 | 0.24-1.18 | 0.12 | 0.48 | 0.22-1.06 | 0.069 |
| FGF-β | 1.01 | 0.81-1.26 | 0.95 | 1 | 0.80-1.25 | 0.98 |
| G-CSF | 0.97 | 0.85-1.12 | 0.69 | 0.97 | 0.85-1.10 | 0.62 |
| GM-CSF | 1.30 | 0.67-1.58 | 0.89 | 1 | 0.65-1.53 | 1 |
| IL-1β | 1.03 | 0.71-1.50 | 0.87 | 1.09 | 0.75-1.58 | 0.65 |
| IL-1-RA | 1.04 | 0.84-1.30 | 0.67 | 0.96 | 0.77-1.18 | 0.67 |
| IL-2 | 0.76 | 0.42-1.37 | 0.36 | 0.71 | 0.40-1.26 | 0.24 |
| IL-4 | 0.91 | 0.56-1.47 | 0.69 | 0.97 | 0.61-1.54 | 0.89 |
| IL-6 | 1.26 | 0.90-1.77 | 0.18 | 1.19 | 0.86-1.65 | 0.29 |
| IL-7 | 0.90 | 0.75-1.09 | 0.28 | 0.92 | 0.77-1.11 | 0.40 |
| IL-8 | 1.05 | 0.73-1.52 | 0.78 | 0.89 | 0.62-1.29 | 0.54 |
| IL-9 | 1.83 | 0.72-4.68 | 0.21 | 1.55 | 0.61-3.92 | 0.36 |
| IL-10 | 1.04 | 0.69-1.58 | 0.84 | 1.02 | 0.68-1.52 | 0.92 |
| IL-12 | 0.98 | 0.62-1.56 | 0.93 | 0.96 | 0.61-1.53 | 0.88 |
| IL-13 | **0.52** | **0.33-0.81** | **0.004**[a] | 0.57 | 0.37-0.87 | 0.008 |
| IL-17 | 0.99 | 0.74-1.31 | 0.92 | 1.01 | 0.77-1.33 | 0.94 |
| IP-10 | 2.13 | 1.04–4.36 | 0.04 | 1.74 | 0.85-3.53 | 0.13 |
| MCP-1 | 1.03 | 0.72-1.46 | 0.88 | 1.1 | 0.80-1.53 | 0.55 |
| MIP-1α | 1.46 | 0.92-2.33 | 0.11 | 1.11 | 0.69-1.78 | 0.66 |
| MIP-1δ | 4.61 | 1.08–19.73 | 0.039 | 3.57 | 0.80-15.91 | 0.095 |
| PDGF-BB | 1.15 | 0.95-1.40 | 0.14 | 1.16 | 0.95-1.42 | 0.14 |
| RANTES | 2.17 | 1.20-3.94 | 0.01 | 1.84 | 1.01–3.32 | 0.045 |
| TNF-α | 1.07 | 0.89-1.29 | 0.48 | 1.10 | 0.91-1.32 | 0.34 |
| VEGF | 1.02 | 0.83-1.24 | 0.87 | 1.03 | 0.85-1.26 | 0.75 |

ILD – interstitial lung disease; FGF – fibroblast growth factor, G-CSF – granulocyte colony stimulating factor, GM-CSF – granulocyte-macrophage colony stimulating factor, IL – interleukin, IL-1-RA – interleukin-1 receptor antagonist, IP-10 – interferon gamma induced protein 10, MCP-1 – monocyte chemoattractant protein 1, MIP – macrophage inflammatory protein, PDGF – platelet derived growth factor, RANTES – regulated upon activation, normal T cell expressed and presumably secreted, TNF – tumor necrosis factor, VEGF – vascular endothelial growth factor.
[a] Significant at FDR 10 %.

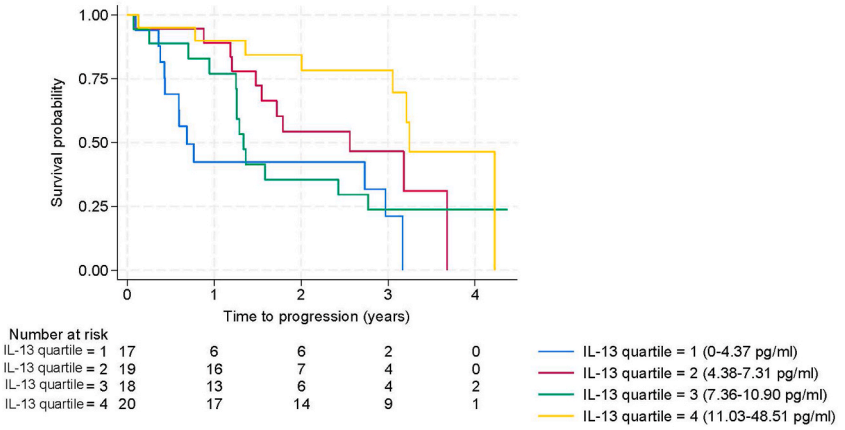


**Fig. 1.** Kaplan-Meier Survival Estimates for IL-13 level (natural log-transformed) by quartiles IL-13 – interleukin-13; HR - hazard ratio.

**Table 4**
Association of baseline variables with lung disease progression in multivariable model.

| Variable | HR | 95 % CI | p |
|---|---|---|---|
| GAP Index | 1.51 | 1.19-1.93 | 0.001 |
| IL-13 | 0.41 | 0.25-0.68 | <0.001 |
| IL-1β | 1.67 | 1.06-2.63 | 0.028 |
| IPAF classification | 3.25 | 1.26-8.40 | 0.015 |

HR – hazard ratio; CI – confidence interval; GAP – Gender, Age, Physiology; IL – interleukin; IPAF – interstitial pneumonia with autoimmune features.

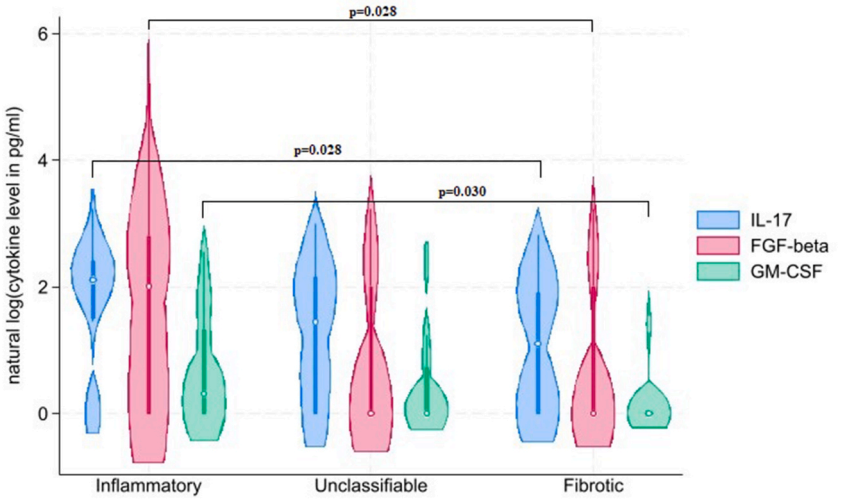


**Fig. 2.** Significant cytokine level (natural log-transformed) differences between ILD subtypes ILD – interstitial lung disease; FGF – fibroblast growth factor; GM-CSF – granulocyte-macrophage colony stimulating factor; IL – interleukin.

**Table 5**
Cytokine level differences between ILD subgroups (inflammatory, fibrotic, and unclassifiable).

| Cytokine | ANOVA | IPF vs RD-ILD/cHP | | IPF vs IPAF/uILD | | RD-ILD/cHP vs IPAF/uILD | |
|---|---|---|---|---|---|---|---|
| | p | Mean difference[a] | p | Mean difference[a] | p | Mean difference[a] | p |
| Eotaxin | 0.060 | 0.15 | 0.32 | −0.13 | 0.46 | −0.28 | **0.049** |
| FGF-β | **0.026** | −0.96 | **0.028** | −0.09 | 0.97 | 0.86 | 0.098 |
| G-CSF | 0.32 | −0.87 | 0.29 | −0.28 | 0.89 | 0.59 | 0.64 |
| GM-CSF | **0.040** | −0.51 | **0.030** | −0.20 | 0.59 | 0.30 | 0.36 |
| IL-1β | 0.60 | −0.20 | 0.58 | −0.13 | 0.83 | 0.08 | 0.94 |
| IL-1-RA | 0.40 | −0.502 | 0.39 | −0.33 | 0.69 | 0.17 | 0.92 |
| IL-2 | 0.30 | −0.27 | 0.27 | −0.14 | 0.72 | 0.13 | 0.79 |
| IL-4 | 0.19 | −1.71 | 0.20 | −1.11 | 0.51 | 0.26 | 0.96 |
| IL-6 | 0.25 | −1.47 | 0.31 | −0.21 | 0.98 | 1.13 | 0.49 |
| IL-7 | 0.50 | −1.26 | 0.42 | −0.33 | 0.94 | 0.62 | 0.81 |
| IL-8 | 0.46 | −1.38 | 0.35 | −0.25 | 0.97 | 0.74 | 0.74 |
| IL-9 | 0.11 | −2.65 | **0.022** | −0.17 | 0.98 | 1.58 | 0.25 |
| IL-10 | 0.54 | −0.68 | 0.77 | −1.24 | 0.43 | −0.40 | 0.91 |
| IL-12 | **0.036** | −2.08 | 0.093 | −0.40 | 0.91 | 1.36 | 0.36 |
| IL-13 | 0.94 | 0.34 | 0.94 | 0.055 | 1 | −0.53 | 0.86 |
| IL-17 | **0.040** | −2.57 | **0.028** | −0.79 | 0.71 | 1.59 | 0.25 |
| IP-10 | 0.22 | −1.42 | 0.33 | −1.50 | 0.29 | −0.28 | 0.96 |
| MCP-1 | 0.84 | −0.44 | 0.90 | −0.62 | 0.81 | −0.12 | 0.99 |
| MIP-1α | 0.94 | −0.42 | 0.91 | −0.80 | 0.70 | −0.39 | 0.92 |
| MIP-1δ | 0.42 | −1.65 | 0.23 | −0.28 | 0.96 | 1.21 | 0.44 |
| PDGF-BB | 0.56 | −1.36 | 0.36 | −0.64 | 0.80 | 0.77 | 0.72 |
| RANTES | 0.15 | −2.31 | 0.054 | −0.37 | 0.93 | 1.53 | 0.28 |
| TNF-α | 0.61 | −2.09 | 0.092 | −1.08 | 0.53 | 0.96 | 0.60 |
| VEGF | 0.13 | −1.80 | 0.17 | −1.60 | 0.25 | 0.23 | 0.97 |

ILD – interstitial lung disease; ANOVA – analysis of variance; IPF – idiopathic pulmonary fibrosis; RD – rheumatic disease; cHP – chronic hypersensitivity pneumonitis; IPAF – interstitial pneumonia with autoimmune features; uILD – unclassifiable ILD; FGF – fibroblast growth factor; G-CSF – granulocyte colony stimulating factor; GM-CSF – granulocyte-macrophage colony stimulating factor; IL – interleukin; IL-1-RA – interleukin-1 receptor antagonist; IP-10 – interferon gamma induced protein 10; MCP-1 – monocyte chemoattractant protein 1; MIP – macrophage inflammatory protein; PDGF – platelet derived growth factor; RANTES – regulated upon activation, normal T cell expressed and presumably secreted; TNF – tumor necrosis factor; VEGF – vascular endothelial growth factor.

[a] By Wilcoxon signed-rank test.

1.08–21.85, p = 0.039) but not when adjusted for GAP. Interestingly, multiple cytokines that did not reach statistically significant association with time to progression when analysis was unadjusted (FGF-β, IL-2, IL-8, IL-12, and MCP-1), were associated with longer time to progression when adjusted for GAP (Appendix table 7).

Within the fibrotic group, increasing IL-13 level was significantly associated with longer time to progression (HR 0.45, 95%CI 0.22–0.90, p = 0.025) while increasing TNF-α was associated with shorter time to progression (HR 1.48, 95%CI 1.02–2.15, p = 0.041)

in unadjusted analysis. When adjusted for GAP, neither IL-13 nor TNF-α were significantly associated with time to progression. However, eotaxin and IL-1-RA, while not significantly associated with time to progression in unadjusted analysis, were significantly associated with longer time to progression when adjusted for GAP (Appendix Table 8).

## 4. Discussion

We aimed to investigate the role of cytokines in predicting outcomes and categorizing ILD. We found that higher IL-13 levels were associated with ILD stability in our cohort, and FGF-β, GM-CSF, and IL-17 differentiated fibrotic ILD types (such as IPF) from inflammatory driven ILDs (cHP and RD-ILD). These findings are currently hypothesis-generating and need to be validated in larger independent cohorts. If validated, IL-13 may offer prognostic information, while FGF-β, GM-CSF, and IL-17 may aid in detecting inflammatory etiology in cases of patients with uILD or IPAF. Finally, we found that higher IL-1-RA was associated with faster progression in inflammatory ILD group, whereas high IP-10 and TNF-α were associated with faster progression in unclassifiable and fibrotic group, respectively. Higher IL-13 was associated with longer time to progression in all ILD groups, although this observation only reached statistical significance in IPF.

The role of IL-13 in the ILD clinical course has previously been debated, with the primary paradigm suggesting the pro-fibrotic role of Th2 immunity [30]. In a study by Versace et al., patients with systemic sclerosis [SSc]-ILD had higher serum IL-13 levels which corresponded to lower pulmonary function [31], while Hussein et al. reported that IL-13 is positively correlated with fibrosis score and pro-fibrotic biomarkers in a cohort of RA-ILD patients [32]. In contrast, our results suggest that patients with high IL-13 levels tend to be protected from ILD progression for longer duration, possibly via Th2 mediated homeostatic mechanism. Matsuno et al., reported on GATA3 overexpression being protective from the development of hypersensitivity pneumonitis in murine model via shifting of Th2/Th1 balance towards Th2; high IL-13 level was observed [33]. Interestingly, phase 2 IPF studies investigating IL-13 blockade have failed to demonstrate benefit over 52 weeks of treatment [34,35], also arguing against the notion that IL-13 is pathogenic in IPF. Furthermore, a recent study by Watson et al., has found that the antifibrotic nintedanib acts in part by promoting IL-4/IL-13-dependent homeostatic macrophages, thus enhancing tissue repair [36]. There results emphasize the possible role of Th2 immunity as being protective against ILD progressive fibrosis. In our cohort, we found that elevated IL-13 levels were associated with longer time to progression in several ILD subtypes, suggesting that this mechanism may be preserved regardless of etiology.

We attempted to identify circulating biomarkers differentiating ILD subtypes. Currently, ILD classification hinges on identification of etiology and invasive testing, such as biopsy and bronchoscopy. In cases when a patient is unable to undergo such testing or results are conflicting, the patient may be labeled as having unclassifiable disease, which presents significant difficulties in diagnosis and management. We found that some pro-inflammatory cytokines FGF-β, GM-CSF, and IL-17 were elevated in inflammatory subtype of ILD as compared to IPF. This suggests that an inflammatory profile may be established, which may be utilized for assigning an etiology in poorly characterized cases of IPAF and uILD.

Other studies have also attempted to find biomarkers which would allow differentiation between subtypes. Kameda et al., found that CXCL9, CXCL10, and CXCL11 levels were highest in RD-ILD, with levels in IPAF being lower and in IPF lower still [18]; however, we did not find significant differences in IP-10 (also known as CXCL10) in our cohort between ILD subtypes. CXCL10 binds to C-X-C receptor 3 and acts as a pro-inflammatory driver by recruiting T-helper and cytotoxic T cells to the site of inflammation in the lung [18]. It is possible that the ongoing inflammation was less active in our patient population than in the study by Kameda et al. [18], and this, coupled with smaller sample size, precluded us from finding similar results. Liang et al. found that CXCR2-CXCL1 axis was activated in IPAF, with higher CXCL1 levels in IPAF than non-IPAF interstitial pneumonia [37]; CXCL1 was not measured in our cohort. White et al., identified a signature of osteopontin, MMP-7, and SP-D combination differentiating IPF from non-IPF ILD [19]. We attempted to identify cytokine signatures within each ILD subtype, but were unable to find differences beyond FGF-β, GM-CSF, and IL-17, possibly owing to small sample size. However, other cytokines measured in our study may be conserved in ILD pathogenesis, regardless of type. Kameda et al., did not identify significant differences in IL-6, IL-8, IL-10, or TNF-α [18], with De Lauretis et al. supporting the notion of IL-6, IL-8, IL-10, and VEGF being conserved biomarkers in SSc-ILD and IPF [12]; additionally, RANTES did not differ significantly in bronchoalveolar lavage fluid of patients with RD-ILD and IPF in another study [38], supporting this hypothesis. Nevertheless, the finding of significant differences in FGF-β, GM-CSF, and IL-17 between inflammatory and fibrotic ILD subtypes in our cohort has potential diagnostic implications for IPAF and uILD. Notably, having IPAF classification was associated with increased hazard of progression in multivariable analysis, which could potentially be due to difficulty with diagnosis and management decision, emphasizing the importance of objectively differentiating between subtypes in ILD [39]. Targeted studies on this patient population can clarify this.

We evaluated for cytokine signatures associated with time to progression within each ILD subtype. Due to small sample size, we had to combine ILD by presumed mechanism into more broad subtypes (inflammatory, unclassifiable, and fibrotic). We did find trends of IL-13 being associated with longer time to progression in the subtypes, although the association was only statistically significant in the fibrotic subtype. We also found that the subtypes had different cytokines associated with progression, suggesting different mechanisms of damage and repair beyond those that might be IL-13-mediated.

Our study has several strengths. First, patients with ILD were classified utilizing multidisciplinary approach, with RD-ILD, IPAF, and uILD patients additionally reviewed by rheumatologist and pulmonologist. This approach may have reduced the possibility of misclassification bias. Despite a small sample size, we were able to identify trends for IL-13 being associated with stability in individual ILD subtypes, suggesting IL-13 may be a relevant marker of disease stability in ILD. Third, we employed several types of analyses to investigate association of cytokines with time to progression, all of which revealed statistical significance of the association of IL-13 with time ro progression.

Our study has limitations. First, a small sample size may have precluded us from finding significant results for other cytokines. For example, MIP-1δ and RANTES showed an association with time to progression; which did not remain significant after adjustment for GAP (in the case of MIP-1δ) or for multiple comparisons at 10 % FDR (for either MIP-1δ or RANTES). However, RANTES may be a plausible predictive cytokine in ILD. RANTES plays a role in T-cell activation and has been hypothesized to contribute to the inflammatory cycle [40]. RANTES levels and mRNA expression were found to be high in BALF of patients with sarcoidosis, RD-ILD, and IPF [38]. Despite this, RANTES has not been identified as a consistent marker of disease progression in ILD. A larger sample size could have allowed for more robust association measurement in our study.

Additionally, small number of patients within individual ILD subtypes prevented us from being able to adjust for confounders when evaluating association of cytokines with time to progression. We were unable to find meaningful cytokine differences between types, preventing us from identifying a reliable cytokine signature within each type. We combined the individual subtypes into broader groups (inflammatory, unclassifiable, and fibrotic), but it is possible that those groups were actually a mix of the subtypes due to somewhat arbitrary nature of the original classification, preventing us from identifying other significant cytokine differences.

Furthermore, the cytokines tested were selected based on availability of the assay which would test the range of cytokines most frequently implicated in ILD and various RDs [20,41–43]. It is possible that other cytokines which were not tested would be found to have meaningful associations with disease behavior and types.

Finally, this was a retrospective study which resulted in missing data and limited our conclusions to associations. We were unable to utilize serial imaging or symptom assessments in our definition for progression due to variable follow up and inconsistent timing of repeat chest imaging. This could have led to misclassification bias in our definition of progression. We did not repeat cytokine measurements over time so we cannot infer on changes in cytokine levels with change in clinical course. We could not control for treatment effect on the outcome due to small sample size; however, we only analyzed patients that were not treated at time of blood collection. Also, we did not have an independent cohort to replicate our results. Therefore, these results should be considered hypothesis-generating and need to be validated in larger studies.

In summary, we identified that higher IL-13 levels were associated with longer time to progression of ILD in our cohort. This is relevant as it provides a possible early biomarker for patients whose disease is less likely to progress as well as provide potential insight into protective mechanisms in ILD. While we were unable to identify cytokine profiles which would reliably differentiate between ILD subtypes, we did find that higher FGF-β, GM-CSF, and IL-17 levels were present in cHP and RD-ILD than in IPF, a finding which could be utilized in cases of unclassifiable cases or IPAF. The findings are intriguing and may provide support for clinical utilization of cytokine panel testing in ILD and rheumatological clinics. Cytokines may potentially be incorporated into testing when evaluating patients with ILD to help assess the prognosis and etiology, although further large scale studies assessing these biomarkers are needed. Finally, our results suggest that different ILD subtypes may progress via different mechanisms and more research is needed investigating exact mechanisms of damage in individual ILD subtypes.

## 5. Conclusions

Further research is warranted to investigate cytokine levels within progressive and non-progressive ILD phenotypes with the goal of identifying biomarkers predicting progression and elucidating underlying pathogenesis. Furthermore, investigations of biomarker differences between ILD subtypes is important, as accurate classification of ILD has clinical implications for treatment and management. Future larger scale, prospective studies may help address both of these goals.

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## Ethics approval

This study was conducted in accordance with the Declaration of Helsinki, and approved by University of Texas Southwestern institutional review board (protocol #092017-007).

## Consent to participate

All patients provided written informed consent as part of research blood draw for participation in this study.

## Consent to publish

Not applicable as no individual person's data is contained in this manuscript.

## Data availability

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to

privacy restrictions.

## CRediT authorship contribution statement

**Elena K. Joerns:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David Karp:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Song Zhang:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Jeffrey A. Sparks:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Traci N. Adams:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Una E. Makris:** Writing – review & editing, Methodology, Conceptualization. **Chad A. Newton:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32118.

## References

[1] C.J. Ryerson, H.R. Collard, Update on the diagnosis and classification of ILD, Curr. Opin. Pulm. Med. 19 (5) (2013) 453–459.
[2] G. Raghu, et al., Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline, Am. J. Respir. Crit. Care Med. 198 (5) (2018) e44–e68.
[3] V. Cottin, et al., Presentation, diagnosis and clinical course of the spectrum of progressive-fibrosing interstitial lung diseases, Eur. Respir. Rev. 27 (150) (2018).
[4] M. O'Callaghan, F. Bonella, C. McCarthy, Unclassifiable, or simply unclassified interstitial lung disease? Curr. Opin. Pulm. Med. 27 (5) (2021) 405–413.
[5] A. Fischer, et al., An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features, Eur. Respir. J. 46 (4) (2015) 976–987.
[6] R.C. Chambers, P.F. Mercer, Mechanisms of alveolar epithelial injury, repair, and fibrosis, Ann Am Thorac Soc 12 (Suppl 1) (2015) S16–S20. Suppl 1.
[7] S. Guler, et al., Phenotyping by persistent inflammation in systemic sclerosis associated interstitial lung disease: a EUSTAR database analysis, Thorax 78 (12) (2023) 1188–1196.
[8] T.M. Maher, et al., An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study, Lancet Respir. Med. 5 (12) (2017) 946–955.
[9] A.A. van Batenburg, et al., Telomere shortening and DNA damage in culprit cells of different types of progressive fibrosing interstitial lung disease, ERJ Open Res 7 (2) (2021).
[10] G.C. Goobie, et al., Air pollution and interstitial lung diseases: defining epigenomic effects, Am. J. Respir. Crit. Care Med. 202 (9) (2020) 1217–1224.
[11] S. Alqalyoobi, et al., Circulating plasma biomarkers of progressive interstitial lung disease, Am. J. Respir. Crit. Care Med. 201 (2) (2020) 250–253.
[12] A. De Lauretis, et al., Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis, J. Rheumatol. 40 (4) (2013) 435–446.
[13] G. Raghu, et al., Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline, Am. J. Respir. Crit. Care Med. 205 (9) (2022) e18–e47.
[14] K.R. Flaherty, et al., Nintedanib in progressive fibrosing interstitial lung diseases, N. Engl. J. Med. 381 (18) (2019) 1718–1727.
[15] M. Xue, et al., Evaluation of the diagnostic efficacies of serological markers KL-6, SP-A, SP-D, CCL2, and CXCL13 in idiopathic interstitial pneumonia, Respiration 98 (6) (2019) 534–545.
[16] N. Ishikawa, et al., Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases, Respir Investig 50 (1) (2012) 3–13.

[17] J. Massagué, TGFβ signalling in context, Nat. Rev. Mol. Cell Biol. 13 (10) (2012) 616–630.
[18] M. Kameda, et al., CXCL9, CXCL10, and CXCL11; biomarkers of pulmonary inflammation associated with autoimmunity in patients with collagen vascular diseases-associated interstitial lung disease and interstitial pneumonia with autoimmune features, PLoS One 15 (11) (2020) e0241719.
[19] E.S. White, et al., Plasma surfactant protein-D, matrix metalloproteinase-7, and osteopontin Index distinguishes idiopathic pulmonary fibrosis from other idiopathic interstitial pneumonias, Am. J. Respir. Crit. Care Med. 194 (10) (2016) 1242–1251.
[20] J.J. O'Shea, A. Ma, P. Lipsky, Cytokines and autoimmunity, Nat. Rev. Immunol. 2 (1) (2002) 37–45.
[21] P. Montero, et al., Role of JAK/STAT in interstitial lung diseases; molecular and cellular mechanisms, Int. J. Mol. Sci. 22 (12) (2021).
[22] I. Campo, M. Zorzetto, F. Bonella, Facts and promises on lung biomarkers in interstitial lung diseases, Expert Rev Respir Med 9 (4) (2015) 437–457.
[23] A.S. Jee, et al., Review: serum biomarkers in idiopathic pulmonary fibrosis and systemic sclerosis associated interstitial lung disease - frontiers and horizons, Pharmacol. Ther. 202 (2019) 40–52.
[24] M. Aringer, et al., European League against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus, Ann. Rheum. Dis. 78 (9) (2019) 1151–1159, 2019.
[25] F. van den Hoogen, et al., 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative, Ann. Rheum. Dis. 72 (11) (2013) 1747–1755.
[26] C.H. Shiboski, et al., 2016 American college of rheumatology/European league against rheumatism classification criteria for primary sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts, Arthritis Rheumatol. 69 (1) (2017) 35–45.
[27] D. Aletaha, et al., 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative, Arthritis Rheum. 62 (9) (2010) 2569–2581.
[28] I.E. Lundberg, et al., European League against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups, Ann. Rheum. Dis. 76 (12) (2017) 1955–1964, 2017.
[29] G.H. Green, P.J. Diggle, On the operational characteristics of the Benjamini and hochberg false discovery rate procedure, Stat. Appl. Genet. Mol. Biol. 6 (2007) Article27.
[30] R.L. Gieseck 3rd, M.S. Wilson, T.A. Wynn, Type 2 immunity in tissue repair and fibrosis, Nat. Rev. Immunol. 18 (1) (2018) 62–76.
[31] A.G. Versace, et al., IL-13 and IL-33 serum levels are increased in systemic sclerosis patients with interstitial lung disease, Front. Med. 9 (2022) 825567.
[32] M.S. Hussein, et al., Identification of serum interleukin-13 and interleukin-13 receptor subunit expressions: rheumatoid arthritis-associated interstitial lung disease, Int J Rheum Dis 24 (4) (2021) 591–598.
[33] Y. Matsuno, et al., Overexpression of GATA-3 protects against the development of hypersensitivity pneumonitis, Am. J. Respir. Crit. Care Med. 176 (10) (2007) 1015–1025.
[34] G. Raghu, et al., SAR156597 in idiopathic pulmonary fibrosis: a phase 2 placebo-controlled study (DRI11772), Eur. Respir. J. 52 (6) (2018).
[35] T.M. Maher, et al., Phase 2 trial to assess lebrikizumab in patients with idiopathic pulmonary fibrosis, Eur. Respir. J. 57 (2) (2021).
[36] C.K. Watson, et al., Antifibrotic drug nintedanib inhibits CSF1R to promote IL-4-associated tissue repair macrophages, Am. J. Respir. Cell Mol. Biol. 68 (4) (2023) 366–380.
[37] M. Liang, et al., Clinical association of chemokine (C-X-C motif) ligand 1 (CXCL1) with interstitial pneumonia with autoimmune features (IPAF), Sci. Rep. 6 (2016) 38949.
[38] N. Kodama, et al., Expression of RANTES by bronchoalveolar lavage cells in nonsmoking patients with interstitial lung diseases, Am. J. Respir. Cell Mol. Biol. 18 (4) (1998) 526–531.
[39] H.K. Min, et al., Recent advances in the diagnosis and management of interstitial pneumonia with autoimmune features: the perspective of rheumatologists, Korean J Intern Med 36 (3) (2021) 515–526.
[40] P. Conti, M. DiGioacchino, MCP-1 and RANTES are mediators of acute and chronic inflammation, Allergy Asthma Proc. 22 (3) (2001) 133–137.
[41] A. Bergeron, et al., Cytokine profiles in idiopathic pulmonary fibrosis suggest an important role for TGF-beta and IL-10, Eur. Respir. J. 22 (1) (2003) 69–76.
[42] A. Tzouvelekis, et al., Serum biomarkers in interstitial lung diseases, Respir. Res. 6 (2005) 78.
[43] G. Rolla, et al., Th-17 cytokines and interstitial lung involvement in systemic sclerosis, J. Breath Res. 10 (4) (2016) 046013.