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Alveolar cell composition in interstitial lung disease and the development of a pulmonary progressive fibrosing phenotype: a retrospective cohort study

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Abstract

Background Immunological bronchoalveolar lavage (iBAL) is a frequently employed diagnostic tool in interstitial lung disease (ILD). The association between iBAL cellular composition and disease progression remains elusive. We evaluated whether the alveolar cellular composition at initial diagnosis is predictive of the development of progressive pulmonary fibrosis (PPF) in patients with ILD.

Methods A retrospective analysis of 111 patients diagnosed with ILD who underwent iBAL for diagnostic purposes between January 2018 and January 2023 was conducted. The identification of PPF was based on the criteria outlined in the ATS/ERS/JRS/ALAT clinical practice guidelines. Clinical data, pulmonary function tests, radiological imaging, and BAL cellular composition were collected. Groups were compared using the non-parametric Wilcoxon rank sum test. Linear mixed-effect modelling was used to assess the association between baseline cell composition and longitudinal lung function decline.

Results A total of 33.3% of patients exhibited a PPF phenotype. A significant association between baseline iBAL CD4 + and CD8 + T cell percentages and forced vital capacity (FVC) decline within the first year was observed. Other cell types were not associated with ILD progression within one-year of follow-up.

Conclusions CD4 + and CD8 + T cell percentages significantly correlated with FVC changes in patients with fibrotic ILD. No further associations were found between the baseline iBAL cellular profiles and disease progression. These findings suggest that baseline iBAL cellular profiles may hold some promise in predicting fibrotic ILD disease progression. Further (prospective) studies using larger cohorts may be needed to elucidate the association between the cellular composition of iBAL fluid and pulmonary fibrosis progression.

Keywords Bronchoalveolar lavage (BAL), Interstitial lung disease (ILD), Prognosis, Progressive pulmonary fibrosis (PPF), Pulmonary fibrosis (PF)

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Background

Interstitial lung diseases (ILDs) encompass a diverse spectrum of respiratory conditions characterised by interstitial fibrosis and inflammation [1]. Idiopathic pulmonary fibrosis (IPF) emerges as the most prevalent and severe variant within this spectrum, marked by progressive fibrosis, worsening respiratory symptoms, declining respiratory function, and poor survival [2, 3]. Developing progressive pulmonary fibrosis is not limited to IPF and can extend to other ILDs, including other idiopathic interstitial pneumonias (IIPs) (e.g., idiopathic non-specific interstitial pneumonia (iNSIP), connective tissue disease (CTD)-related ILDs, hypersensitivity pneumonitis (HP), drug-induced ILDs, and sarcoidosis) [3, 4]. Approximately 25% of non-IPF ILD patients are estimated to develop a progressive phenotype, according to the PROGRESS study [5]. Due to their clinical and pathogenic similarities to IPF, these patients are referred to as having a progressive pulmonary fibrosis (PPF) phenotype [3, 4].

Amidst the challenges in diagnosing and predicting the rate of decline of PPF, a growing imperative exists for effective diagnostic tools and early intervention strategies. Despite the widely recognised importance of early detection, current PPF diagnosis relies primarily on indicators of advanced disease. These include clinical deterioration, pulmonary function tests (PFTs), and radiological findings that often signify irreversible damage. This approach potentially narrows the window for effective therapeutic interventions, underscoring the need for more proactive diagnostic methods [6].

Immunological bronchoalveolar lavage (iBAL) is a minimally invasive diagnostic procedure and is used to provide direction to the correct ILD diagnosis. During the procedure, alveolar cells are collected by lavage of the alveolar compartment using a flexible bronchoscope. Subsequently, cellular composition is analysed, providing valuable insights for supporting or excluding specific diagnoses [7]. Recent ATS/JRS/ALAT clinical practice guidelines mention that lymphocyte analysis of iBAL cellular contents can play a crucial role in distinguishing fibrotic HP from sarcoidosis and IPF [3, 8]. Additionally, characteristic patterns for eosinophilic pneumonia and sarcoidosis can be discerned through iBAL cellular analysis [9].

A recent retrospective study indicated that the iBAL cellular composition, particularly the lymphocyte percentage, might be correlated with the diagnosis and prognosis of NSIP, idiopathic pleuroparenchymal fibroelastosis, and unclassifiable idiopathic interstitial pneumonia [10]. However, a notable limitation was that participants had received treatment with immunosuppressive agents before bronchoscopy, potentially influencing the iBAL cellular results. Consequently, the genuine correlation between BAL cell composition and the progression of these diseases remains elusive.

This retrospective observational cohort study aims to investigate the association between different alveolar cell compositions obtained through iBAL at initial diagnosis and developing PPF in different subforms of (fibrotic) ILD. We hypothesise that specific cell types contribute to the fibrotic process and that specific iBAL compositions are correlated with the development of a PPF phenotype.

Methods

Study design and patient population

For this retrospective cohort study, we included patients who underwent iBAL for diagnostic purposes between January 2018 and January 2023 at the Amsterdam UMC Centre of Expertise for ILD in the Netherlands (Figure S1, STROBE study flowchart). The final diagnosis of ILD was established according to ATS/ERS/JRS/ALAT guidelines during interdisciplinary meetings with pulmonologists, radiologists, and pathologists [3, 11]. We included patients with IPF as well as those with both non-progressive and progressive ILD.

Patients were excluded in the following cases: (1) without a final definite diagnosis of ILD were excluded, as were patients with a definite diagnosis of an ILD subform in which the risk of developing a PPF phenotype is less well established (e.g., vasculitis, lymphangioleiomyomatosis, pulmonary alveolar proteinosis), (2) those under 18 years of age, (3) patients receiving immunosuppressants at the time of iBAL, and if no baseline chest HRCT was available within one year prior to the iBAL. Patients were also excluded if clinical, consecutive PFT and radiological follow-up data were missing within 1 year after diagnosis or if they developed a malignancy or progressive pulmonary hypertension during this period of follow-up.

Clinical, PFT, bronchoalveolar lavage fluid (BALF) cytology, histology (e.g., cryobiopsy, transbronchial biopsy, surgical resection, explant, or endoscopic ultrasound-guided fine-needle aspiration), and CT imaging data were retrieved from the institution's database and/or electronic medical patient records. The iBAL procedure performed on participants was an integral part of the standard diagnostic care at our ILD centre, as part of MDT discussions to determine the final diagnosis. BALF cytology data included percentages of macrophages, neutrophils, eosinophils, and lymphocytes, with lymphocyte subset analysis when feasible, all of which were documented at baseline. Details on the iBAL procedure can be found in the Supplementary Method section.

PPF was defined as meeting at least two of the following three criteria within one year post-iBAL procedure: (1) worsening respiratory symptoms; (2) an absolute decline in forced vital capacity (FVC) ≥ 5%; or (3) an absolute decline in diffusing capacity of the lung for carbon

monoxide (DLCO) of ≥ 10%, and/or radiological evidence of disease progression in line with ATS/ERS/JRS/ALAT guidelines [3]. Assessment of worsening respiratory symptoms involved extracting patient anamnesis data from medical records, focussing on dyspnoea, cough, and chest pain. Symptoms were categorised as increased, stable, or decreased, excluding alternative explanations such as pulmonary tract infections. Radiological progression was evaluated using clinical CT scans performed within one year after iBAL. The scans were classified according to the absence of fibrotic progression, fibrotic progression, or uncertainty (considered no progression). Fibrotic progression was defined by an increase in the extent and/or severity of traction bronchiectasis, new fine reticulation, increased coarseness of reticular abnormalities, new or intensified honeycombing, and/or augmented lobar volume loss [3]. The one-year focus for follow-up data ensures that the results remain clinically relevant, interpretable, and comparable across participants and as close to the BAL sampling as possible.

Ethical approval

This study was approved by the Medical Ethics Review Committee of Amsterdam University Medical Centre (number 2023.0564) and was performed in accordance with the Declaration of Helsinki and Dutch regulations. Clinical trial number: not applicable.

Statistical analysis

Descriptive statistics were used to summarise patient characteristics with standard summary statistics for distribution. Normally distributed continuous data were presented as mean and standard deviation (SD), whereas non-normally distributed continuous data were expressed as median and interquartile range (IQR). Categorical data were presented as percentages. Group differences were evaluated using appropriate statistical tests like the Mann-Whitney U test or unpaired two-sample t-test.

Differences in the iBAL differential cell count and lymphocyte subsets among ILD subforms were assessed using the non-parametric Kruskal-Wallis test. Post-hoc analysis was conducted with Dunn's multiple comparison test, and a Bonferroni adjustment was applied to reduce the family-wise error rate. The non-progressive and progressive groups were compared using the non-parametric Wilcoxon rank sum test after checking the violation of the Shapiro-Wilk test for normality.

The association between the cell count and lymphocyte subsets at baseline and the dynamic changes in PFT outcomes were analysed using joint model analysis (*JM package*), which combines a linear mixed model and Cox proportional hazards model. Separate models were constructed for the FVC percentage of predicted (%pred) and DLCO percentage of predicted (%pred) as dependent variables. The independent variable was set separately for each differential cell type and lymphocyte subset retrieved during BALF analyses. All linear mixed-effects models were adjusted for the days from iBAL retrieval, incorporating a random intercept for each patient.

All statistical analyses were performed using R statistical software (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria). Figures were created with GraphPad Prism (version 9.0.0, GraphPad Software, Boston, Massachusetts USA) and R statistical software (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria). A significance level of ≤ 0.05 was considered statistically significant.

Results

Baseline characteristics

The study cohort consisted of 111 ILD patients from whom iBAL data at initial diagnosis was available. Baseline characteristics of the total patient cohort are detailed in Table S1. In Table 1, a comparison of baseline characteristics between progressive versus non-progressive ILD patients is shown, whereas in Table S2 also a separation between non-IPF and IPF is presented to provide insight in the different groups. The mean age of the study cohort was 66 (57–72) years, and 63.1% were male. HP was the most common final multidisciplinary team (MDT) diagnosis (31.5%), followed by uILD (18.0%), IPF (16.2%), and iNSIP (11.7%). As illustrated in Figure S1, thirty-seven patients (33.3%) met the criteria for PPF, whereas seventy-four patients (66.6%) exhibited a non-progressive phenotype during the one-year follow-up.

Compared with the non-PPF patients, the most frequently established MDT ILD diagnosis in PPF patients was HP (40.5%), followed by uILD (16.2%) (Table 1). Abnormal counts of macrophages (> 81%), neutrophils (> 2.7%), and lymphocytes (> 17%) in the BALF were observed in 71.2%, 81.8%, and 48.6% of patients, respectively. As shown in Table 2, significant differences in differential cell counts were observed between the different MDT diagnoses. Indeed, the percentages of macrophages ($p = 0.008$) and lymphocytes ($p < 0.001$) were highest in the IPF (79.9%) and HP (27.7%) groups, respectively. Post-hoc analysis elucidated distinct differences in macrophage proportions, with significant differences between IPF and HP ($p = 0.0072$) and IPF and other subforms ($p = 0.0364$). As expected, differences in lymphocyte proportion were evident between IPF and HP ($p = 0.0002$). No further significant differences were observed in other cell types across the different MDT diagnoses. A detailed overview regarding iBAL differential cell count and lymphocyte subsets among various ILD subforms is provided in Table 2 and Figure S2.

Table 1 Baseline patient demographics and clinical characteristics

	Non-Progressive (*n* = 74)	Progressive (*n* = 37)	*P*-value
Age, median [IQR], y	66 [56–72]	68 [62–72]	0.32
Sex			0.63
Female, n (%)	29 (39.2)	12 (32.4)	
Male, n (%)	45 (60.8)	25 (67.6)	
Smoking status			0.95
Never, n (%)	23 (31.1)	11 (29.7)	
Former, n (%)	44 (59.5)	23 (62.2)	
Current, n (%)	7 (9.5)	3 (8.1)	
Ethnicity			0.74
Caucasian, n (%)	53 (71.6)	27 (73.0)	
Other, n (%)	18 (16.2)	8 (7.2)	
Unknown, n (%)	3 (4.1)	2 (5.4)	
Pulmonary function test at baseline			
FVC % pred, mean (SD)	80.5 (17.0)	79.1 (15.3)	0.66
FEV_1 % pred, mean (SD)	81.6 (18.8)	81.00 (17.8)	0.88
D_{LCO} % pred, median [IQR]*	54.0 [47.5–64.0]	53.0 [46.1–59.5]	0.64
iBAL			
Macrophages, median [IQR], %	67.2 [42.2–81.4]	73.0 [34.0–85.0]	0.80
Neutrophils, median [IQR], %	6.6 [3.4–11.0]	6.7 [4.0–13.1]	0.80
Eosinophils, median [IQR], %	2.0 [1.0–3.0]	2.4 [1.0–4.2]	0.52
Lymphocytes, median [IQR], %	17.5 [7.0–34.0]	10.0 [6.0–42.0]	0.77
CD4 + T cells, median [IQR], %*	60.0 [38.0–75.0]	59.0 [44.0–73.0]	0.55
CD8 + T cells, median [IQR], %*	29.0 [17.0–46.0]	25.0 [18.0–40.0]	0.43
NK cells, median [IQR], *	4.0 [2.0–7.0]	3.0 [2.0–6.0]	0.83
B cells, median [IQR], %*	0.7 [0.4–1.0]	0.9 [0.50–1.0]	0.43
Ratio CD4+/CD8+, median [IQR]*	2.0 [0.9–4.6]	2.4 [1.1–3.9]	0.53
Final MDT diagnosis			0.70
IPF, n (%)	14 (18.9)	4 (10.8)	
HP, n (%)	20 (27.0)	15 (40.5)	
Idiopathic NSIP, n (%)	8 (10.8)	5 (13.5)	
Unclassifiable ILD, n (%)	14 (18.9)	6 (16.2)	
Sarcoidosis, n (%)	8 (10.8)	3 (8.1)	
Other^, n (%)	10 (13.5)	4 (10.8)	
Treatment started after iBAL			< 0.001
Wait and see, n (%)	32 (43.2)	6 (16.2)	
Antifibrotics, n (%)	10 (13.5)	3 (8.1)	
Immunosuppressants, n (%)	32 (43.2)	24 (64.9)	
Both antifibrotics and immunosuppressants, n (%)	0 (0)	4 (10.8)	

* Data not available for all participants. Missing data were as follows: DLCO % pred, 7; lymphocyte subtypes, 1. ^ Connective tissue disease ILD, eosinophilic pneumonia, idiopathic lymphoid interstitial pneumonia, organizing pneumonia, pleuroparenchymal fibroelastosis, and smoking-related interstitial lung disease. Abbreviations: % pred = percentage of predicted; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; HP = hypersensitivity pneumonitis; iBAL = immunological bronchoalveolar lavage; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; IQR = interquartile range; MDT = multidisciplinary team; NK = natural killer; NSIP = non-specific interstitial pneumonia

Baseline PFT (Table 1) and differential cell counts (Fig. 1) did not significantly differ between the non-PPF and PPF groups. A notable distinction between PPF and non-PPF patients was observed regarding treatment started in the year after the iBAL, with a higher frequency of "wait and see" strategy (43.2%) in the non-PPF group, whereas the majority of the PPF group received antifibrotic and/or immunosuppressive therapy (83.8%).

Alveolar differential cell count and lymphocyte subsets at initial diagnosis and development of a PPF phenotype

To investigate the association between iBAL cell composition at initial diagnosis and the development of a PPF phenotype, the percentage of each cell type in the non-PPF and PPF groups was compared. As shown in Fig. 1, no significant differences were observed.

Exploring potential differences in differential cell counts and lymphocyte subsets between PPF and

Table 2 Differential cell counts and lymphocyte subsets among ILD subforms

	IPF (*n* = 18)	HP (*n* = 35)*	Idiopathic NSIP (*n* = 13)	Unclassifiable ILD (*n* = 20)	Sarcoidosis (*n* = 11)	Other (*n* = 14)	*P*-value
Macrophages %	79.9 [74.0–88.5]	64.0 [30.0–76.5]	73.0 [42.0–85.0]	72.5 [44.8–86.5]	65.0 [46.5–68.5]	54.1 [27.3–74.5]	0.008
Neutrophils %	8.5 [5.0-15.5]	6.6 [3.5–11.1]	8.6 [3.0–10.0]	8.5 [4.8–13.7]	4.0 [2.0–5.1]	5.0 [2.6–9.5]	0.23
Eosinophils %	1.4 [1.0–2.0]	1.0 [0.0–3.0]	3.0 [0.4–4.0]	0.8 [0.0–3.0]	1.0 [0.0–3.5]	0.1 [0.0–0.9]	0.40
Lymphocytes %	6.8 [4.2–9.7]	27.7 [12.2–56.4]	17.0 [4.6–44.0]	12.0 [5.0–22.3]	27.0 [15.5–33.8]	21.8 [8.5–57.0]	< 0.001
CD4 + T cells %	59.0 [42.3–71.8]	68.0 [51.8–76.5]	48.0 [38.0–60.0]	55.0 [37.8–68.0]	69.0 [37.0–78.5]	42.5 [36.0–68.0]	0.16
CD8 + T cells %	26.5 [17.0–36.5]	24.5 [14.3–34.5]	38.0 [23.0–56.0]	29.0 [20.5–42.8]	24.0 [10.5–56.5]	45.5 [26.8–54.0]	0.24
NK cells %	4.5 [2.0–7.8]	3.5 [2.0–7.0]	3.0 [2.0–9.0]	4.5 [2.0–6.0]	2.0 [1.0–3.0]	4.0 [2.3–6.8]	0.13
B cells %	1.0 [0.4–2.0]	0.8 [0.5–1.0]	1.0 [0.5–1.0]	0.8 [0.5–1.3]	0.7 [0.2–2.0]	0.7 [0.5–1.7]	0.98
Ratio CD4+/CD8+	2.4 [1.1–4.5]	2.7 [1.4–5.7]	1.3 [0.7–2.3]	1.7 [0.8–3.4]	2.8 [0.7–5.5]	1.0 [0.6–2.7]	0.20

Data are presented as the median [interquartile range]. * Data from one patient in the lymphocyte subset analysis were missing. HP = hypersensitivity pneumonitis; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; NK = natural killer; NSIP = non-specific interstitial

non-PPF disease per MDT diagnosis, the Kruskal-Wallis test revealed significance for macrophages and lymphocytes, indicating variation among ILD diagnoses (Fig. 2 and Table S3). However, the subsequent Dunn's test for multiple pairwise comparisons, adjusted with Holm's correction, did not identify any statistically significant differences between specific groups.

iBAL cytology at initial diagnosis and pulmonary function test decline over time

To determine whether there is an association between cell composition at baseline and lung function decline over time, a linear mixed model was used. As shown in Fig. 3a, the percentage of CD8 + T cell was negatively correlated ($P < 0.01$), indicating that an increase in the percentage of CD8 + T cells of 1% corresponded to a decrease in the FVC %pred of -0.24% (95% confidence interval (CI): -0.390, -0.080) during one-year follow-up. In addition, a positive correlation between CD4 + T cells ($P < 0.01$) and FVC was found, where a 1% increase in CD4 + T cells was associated with a 0.22% increase in FVC %pred (CI: 0.072, 0.359). No significant association between a specific cell type and DLCO changes over time was found (Fig. 3b).

Discussion

In this study, we investigated whether the iBAL cell composition at initial ILD diagnosis might be predictive of disease progression and the development of a PPF phenotype within 1 year of follow-up. This study revealed no significant association between specific alveolar cell compositions at baseline and progressive (fibrotic) disease after 1 year in patients with IPF, HP, NSIP, unclassifiable ILD, and sarcoidosis. However, with a small but statistically significant predictive effect, a greater percentage of CD4 + T cells predicts a slightly improved FVC (0.22% increase per 1% increase in CD4 + T cells) after one-year of follow-up, while a greater percentage of CD8 + T cells predicts decline in the FVC (-0.24% per 1% increase in CD8 + T cells).

The diagnostic potential of BALF analysis, particularly iBAL cell analysis, has been extensively explored, as it serves a valuable supporting tool in MDT decision-making for definitive ILD diagnoses. Our retrospective analysis aligns with prior research, which revealed a higher lymphocyte percentage in HP compared to IPF [12, 13]. Additionally, a comprehensive meta-analysis incorporating 42 studies underscored that chronic HP is characterised by an estimated lymphocyte percentage of 43%, in contrast to the 10% reported in IPF [14]. This consistency underscores the representativeness of our cohort within the broader ILD population. Moreover, our study echoes the findings of Sobiecka et al. (2023) by highlighting a significant difference in macrophage proportions between IPF and HP, supporting the distinct diagnostic considerations between fibrotic HP and IPF [13]. While there was an overall difference in macrophage and lymphocyte counts among the different ILD diagnoses, when comparing PPF and non-PPF patients, no significant differences were found (Fig. 1). This suggests that the observed differences might be due to the variability in cell types between the different subforms rather than distinct differences between PPF and non-PPF.

The diagnostic utility of iBAL is subject to significant limitations. This constraint is notably evident in conditions such as HP and uILD, which often present with comparable clinical features. Despite the initial expectation that iBAL would provide clear distinctions, the cellular compositions obtained from these procedures have not revealed sufficiently unique patterns to serve as definitive diagnostic markers distinguishing these disease entities. The similarity in cellular profiles between HP and uILD underscores the complexity of these conditions and highlights the inadequacy of relying solely on iBAL cell composition for a definitive diagnosis. Consequently, while iBAL remains a valuable tool in the diagnostic process, its results must be interpreted within a broader clinical context rather than being used as a standalone diagnostic criterion.

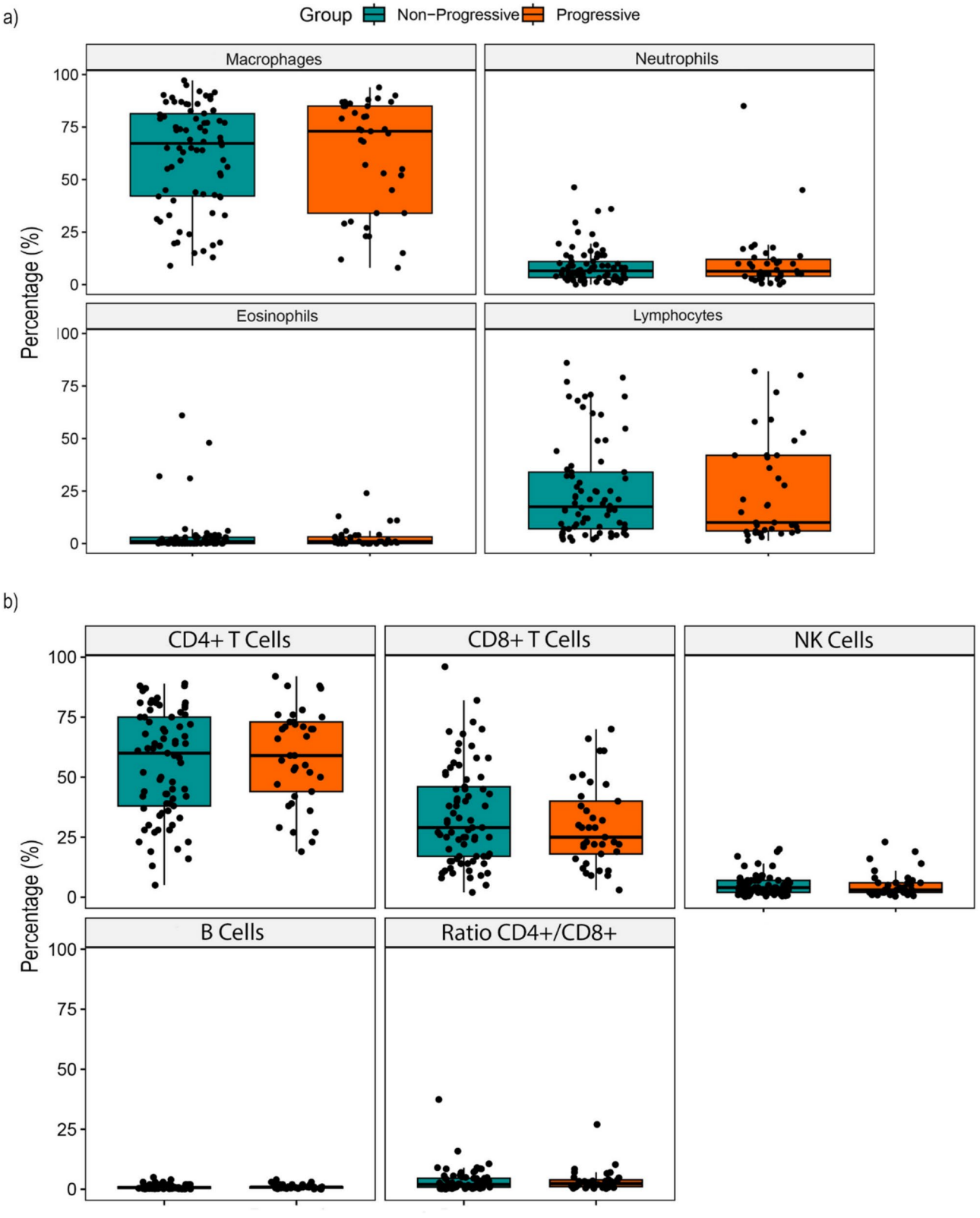

Fig. 1 (**a**) Baseline cell counts and (**b**) Baseline lymphocyte subsets of progressive versus non-progressive interstitial lung disease patients. Data are presented as percentages of cells per cell type. NK = natural killer

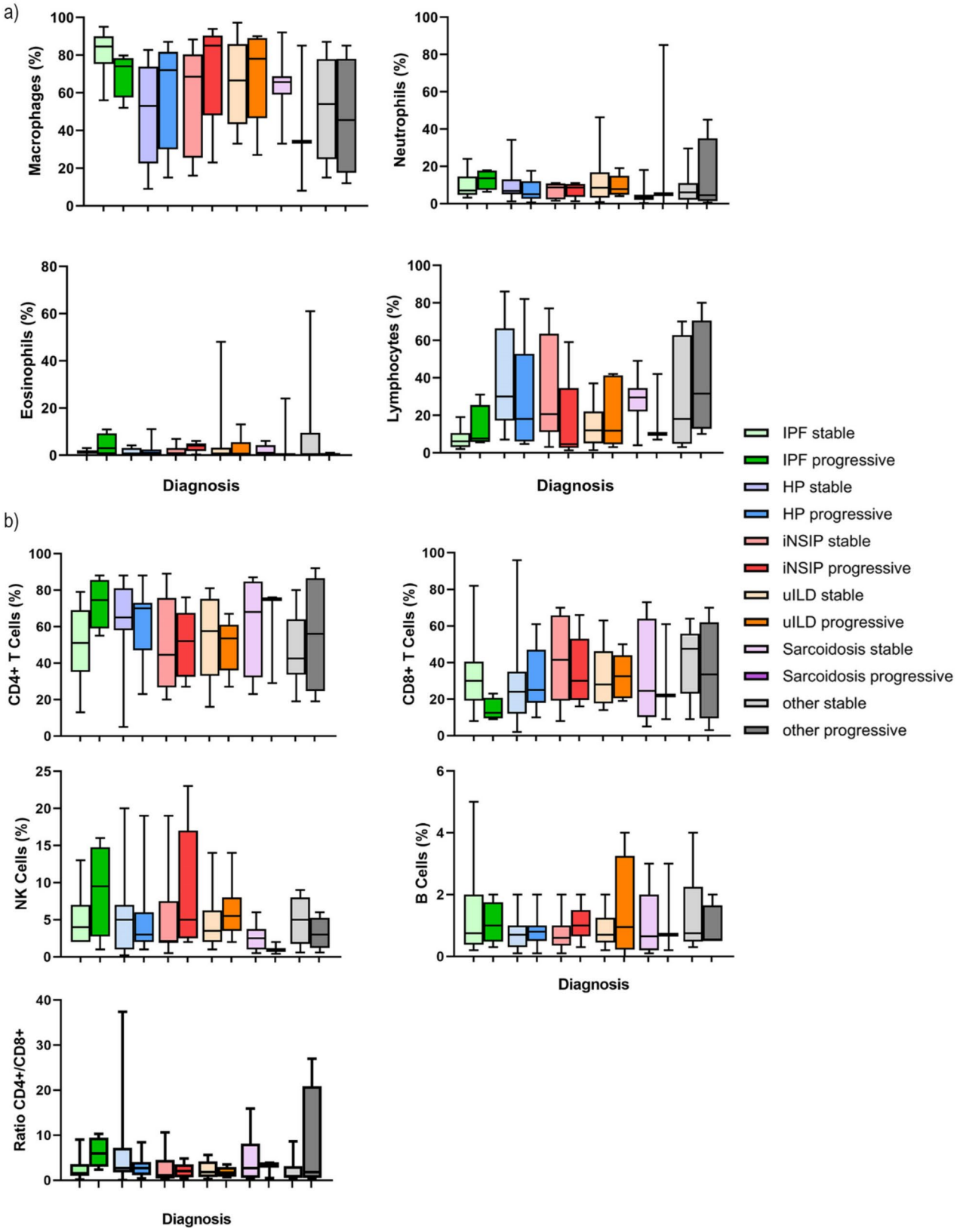

Fig. 2 (**a**) Baseline cell counts and (**b**) Baseline lymphocyte subsets per ILD and progressive and non-progressive groups. Data are presented as percentages per cell type. HP = hypersensitivity pneumonitis; IPF = idiopathic pulmonary fibrosis; iNSIP = idiopathic non-specific interstitial pneumonia; NK = natural killer; uILD = unclassifiable interstitial lung disease

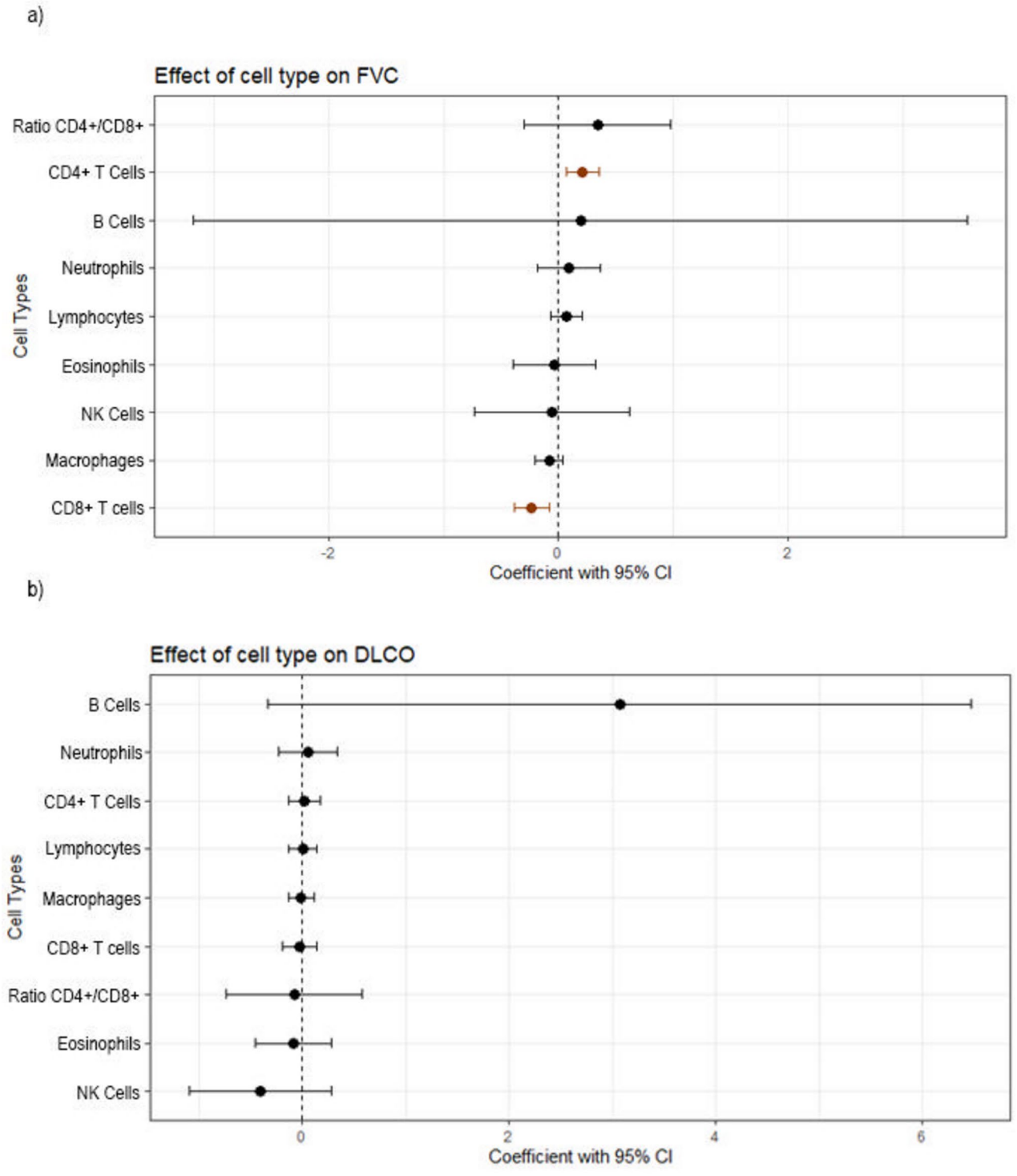

Fig. 3 (**a**) Forest plots of the effect of cell type on FVC change and (**b**) Effect of cell type on DLCO change. Linear mixed-effects model was used to assess the association between iBAL cell type and changes in lung function over time. Both models were corrected for the days from the first and last lung function tests. Data are presented on the Y-axis as all measured BAL differential cells and lymphocyte subsets. The X-axis represents the mean and 95% confidence interval. NK = natural killer

Studies examining the relationship between iBAL cell count and prognosis in ILD patients have yielded mixed results. Our findings align with those of Froidure et al. (2023), who investigated the predictive value of iBAL in IPF patients and found no association between baseline BAL cell counts and the rate of progression [7]. However, other studies have suggested that the iBAL lymphocyte count may have prognostic value in various fibrotic lung diseases, including IPF [15], NSIP [16], and HP [17]. While BAL is not recommended in IPF patients due to the risk of acute exacerbation, in the past it has shown predictive value, with high lymphocyte, neutrophil, and macrophage counts being associated with better survival [15]. The proposed pathophysiological mechanism of IPF suggests a fibroproliferative process rather than a primarily inflammatory process. The lack of a significant inflammatory component in IPF may explain the low lymphocyte counts in the BALF and the poor response of IPF patients to anti-inflammatory agents such as corticosteroids.

Despite a weak correlation, we found a significant association of CD4 + and CD8 + lymphocyte subtypes with FVC (% predicted) decline. Specifically, higher CD4 + T cell levels are associated with an improvement in the FVC over one year, while lower CD8 + T cell levels are linked to a decrease in the FVC after one year. The CD4+/CD8 + ratio in BALF is a commonly used diagnostic marker for sarcoidosis, with an elevated ratio being highly specific for the disease (0.83), although the sensitivity is low (0.70) [18]. However, little is known about its prognostic value in relation to the development of progressive fibrosis. Watase et al. showed that CD8 + T cells in BALF were an independent diagnostic predictor for progressive ILD but found no role for CD4 + T cells [19]. Interestingly, in chronic obstructive pulmonary disease (COPD), a decrease in CD4 + T cells and an increase in CD8 + T cells in the blood may be indicative of disease deterioration [20], although it remains elusive whether this decline is associated with the fibrotic responses observed in COPD. Factors such as smoking and disease severity can influence the BAL CD4+/CD8 + count [18], which further complicates the interpretation of how CD4 + and CD8 + T cells are associated with progression. The pathophysiology of progressive fibrosis in ILD is multifactorial, involving not only inflammation but also other processes, which may account for the variability in these findings.

Our study has several limitations that need to be considered. First, the retrospective design introduced constraints. BAL procedure was part of standard ILD care and only patients with an indication for iBAL were included which may have influenced the results. Also, the relatively small sample size, compounded by the exclusion of participants who passed away from progressive disease and lacked one-year follow-up data, may have introduced selection bias. This bias could potentially underestimate the number of patients with progressive phenotypes in our cohort.

Second, our focus on fibrotic progression was hindered by the lack of uniform radiological data at follow-up, preventing precise assessment of fibrotic progression in all participants. Additionally, the timing of the CT scans varied among patients, with some scans conducted after three months and others after one year, introducing further variability in our assessment. CT scans were evaluated only for fibrotic progression; nonetheless, symptoms and PFT do not distinguish between inflammatory progression and fibrotic progression. However, the PPF phenotype can also be diagnosed based on clinical and lung function deterioration, which are often the first symptoms of the condition [21]. We did find a higher percentage of progressive participants in our than in the ILD population (33.3% versus 13–40%) [22]. This could potentially be explained by the fact that our centre is an expert and referral centre and handles more complicated progressive cases.

Third, our study relied on percentages rather than absolute cell counts and lymphocyte subsets due to the unavailability of absolute values for a significant portion of the patient cohort. While percentages are commonly used in iBAL research, incorporating absolute numbers could have provided a more comprehensive representation of specific ILD subforms, enhancing the depth of our analyses.

Finally, our study did not consider the impact of immunosuppressive and antifibrotic medications on disease progression during the follow-up period. These therapies are known to influence PFT outcomes, potentially confounding our results. The disease could have been progressive at the time of diagnosis, and further progression could be stopped or slowed down with medication. The linear mixed model was not adjusted for treatment effects because of the many different treatment modalities.

Conclusion

In summary, our study contributes to the understanding of the clinical relevance of iBAL cytology in ILDs and underscores the nuanced interplay of cell subsets in ILDs, providing valuable information for diagnostic considerations and furthering our understanding of the underlying pathogenic mechanisms. We could not link alveolar cell composition at initial diagnosis to the development of the PPF phenotype within one year of follow-up. These findings suggest that baseline iBAL cellular profiles may not be reliable predictors of disease progression. Prospective studies are needed to explore other biomarkers for the prediction of a progressive phenotype in ILD patients.

Abbreviations

%pred	percentage of predicted
ALAT	Latin American Thoracic Association
ATS	American Thoracic Society
BALF	Bronchoalveolar Lavage Fluid
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CTD	Connective Tissue Disease
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
ERS	European Respiratory Society
FVC	Forced Vital Capacity
HP	Hypersensitivity Pneumonitis
HRCT	High-Resolution Computed Tomography
iBAL	Immunological Bronchoalveolar Lavage
ILD	Interstitial Lung Disease
iNSIP	Idiopathic Non-Specific Interstitial Pneumonia
IPF	Idiopathic Pulmonary Fibrosis
IIP	Interstitial Pneumonias
IQR	Interquartile Range
JRS	Japanese Respiratory Society
MDT	Multidisciplinary Team
PFT	Pulmonary Function Test
PPF	Progressive Pulmonary Fibrosis
SD	Standard Deviation
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
UMC	University Medical Centre
uILD	Unclassifiable Interstitial Lung Disease

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03236-3.

Supplementary Material 1

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Author contributions

IS, KK, and MH created the database and collected the data. BB, KK, MH, and IS discussed included participants. IS analysed the data and wrote the manuscript. SZ and LB helped IS with the data analyses. BB and EN were involved in the writing of the manuscript; AM and EN supervised the project; JD initiated the study, supervised the data analysis, result interpretation, and manuscript writing. All authors critically read and reviewed the manuscript.

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Data availability

The data that support the findings of this study are included within the article (and its supplementary material).

Declarations

Ethics approval and consent to participate

The clinical protocol was reviewed by the medical ethics committee at the Amsterdam University Medical Centre and was determined to meet the criteria for a waiver of informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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