
Patterns of bronchoalveolar lavage in fibrotic and non-fibrotic interstitial lung diseases – a multicenter analysis

Received: 12 November 2025

Accepted: 27 April 2026

Published online: 30 April 2026

Cite this article as: Gershman E., Freund O., Wand O. *et al.* Patterns of bronchoalveolar lavage in fibrotic and non-fibrotic interstitial lung diseases – a multicenter analysis. *BMC Pulm Med* (2026). <https://doi.org/10.1186/s12890-026-04321-z>

Evgeni Gershman, Ophir Freund, Ori Wand, Sonia Schneer, Jazmin Bloch, Tzlil Hershko, Eyal Kleinhendler, Doron Cohn-Schwartz, Yitzhak Hadad, Galit Aviram, Yochai Adir, David Shitrit, Amir Bar-Shai & Avraham Unterman

We are providing an unedited version of this manuscript to give early access to its findings. Before final publication, the manuscript will undergo further editing. Please note there may be errors present which affect the content, and all legal disclaimers apply.

If this paper is publishing under a Transparent Peer Review model then Peer Review reports will publish with the final article.

ARTICLE IN PRESS

Patterns of bronchoalveolar lavage in fibrotic and non-fibrotic interstitial lung diseases - a multicenter analysis

Evgeni Gershman¹, Ophir Freund^{1,2}, Ori Wand³, Sonia Schneer⁴, Jazmin Bloch^{1,5}, Tzlil Hershko^{1,2}, Eyal Kleinhendler^{1,2}, Doron Cohn-Schwartz^{1,2}, Yitzhak Hadad⁶, Galit Aviram⁶, Yochai Adir⁴, David Shitrit⁷, Amir Bar-Shai¹, Avraham Unterman^{1,2}.

¹Institute of Pulmonary Medicine, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel.

²Center of Excellence for Interstitial Lung Diseases, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel.

³Division of Pulmonary Medicine, Barzilai University Medical Center, Ashkelon, The Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

⁴Pulmonary Division, Lady Davis Carmel Medical Center, Faculty of Medicine, The Technion Institute of Technology, Haifa, Israel.

⁵Division of clinical Laboratories, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel.

⁶Department of Radiology, Tel Aviv Sourasky Medical Center, Tel Aviv University, Tel Aviv, Israel.

⁷Pulmonary Department, Meir Medical Center, Tel Aviv University, Kfar Saba, Israel.

Short title: BAL in fibrotic vs non-fibrotic ILD

Keywords: Bronchoalveolar lavage; Interstitial lung disease; Pulmonary fibrosis; Bronchoscopy; Diagnosis.

Corresponding Author:

Avraham Unterman

Center of Excellence for Interstitial Lung Diseases,
Tel Aviv Medical Center,

Weizmann street 6, 6423906, Tel Aviv, Israel.

Email: ramiu@tlvmc.gov.il, +972-3-6972051

ARTICLE IN PRESS

Abstract:**Background:**

Bronchoalveolar lavage (BAL) cellular analysis is recommended as part of the diagnostic workup of interstitial lung diseases (ILD) when diagnosis is unclear. In recent years, ILD have been classified based on the presence or absence of fibrosis. The objective of this study was to compare BAL cellular profiles across multiple ILDs, stratified by fibrosis status.

Methods:

A retrospective multicenter study of consecutive patients who underwent bronchoscopy, including BAL for ILD evaluation, between 2017 and 2022. BAL indices were chosen based on guidelines and prior research. Chest radiologists blinded to clinical data performed radiological assessment. ILD diagnoses were assigned by multi-disciplinary discussion.

Results:

In total, 238 patients with BAL results were included (mean age 60 years, 46% females, 50.4% with fibrotic ILD, 91% with concurrent transbronchial biopsy). BAL lymphocytes >20% and >30% were more prevalent in non-fibrotic compared to fibrotic ILD (36.4% vs. 17.5% and 25.4% vs. 9.2%, $p<0.01$). In multivariate linear regression model, fibrotic disease was associated with lower lymphocyte counts ($\beta=-6.15$, $p=0.007$). Rates of mixed BAL pattern, lymphocytosis, or isolated neutrophils >3% did not distinguish between fibrotic diseases. On computed tomography, BAL lymphocytosis was not associated with ground-glass opacities or mosaic attenuation in both fibrotic and non-fibrotic groups, while honeycombing was associated with a lower rate of elevated (>4.5%) BAL neutrophils (71% vs. 91%, $p=0.018$).

Conclusion:

Specific patterns in BAL differential were uncommon in fibrotic ILD and were not different among its subtypes. As a stand-alone modality, BAL seems to have a limited utility in fibrotic ILD.

Background:

Interstitial lung diseases (ILD) are a heterogeneous group of parenchymal disorders of various etiologies. The diagnosis of specific ILD subtypes can be challenging due to their variable features, which are often not pathognomonic and may overlap between entities (1). The diagnostic workup for ILD requires a multidisciplinary approach, including radiological, cytological, and pathological analyses (2,3). In cases of diagnostic uncertainty, bronchoscopy may play a role in the workup of ILD (4,5), with several technical modalities available, including bronchoalveolar lavage (BAL), mediastinal lymph node sampling, and transbronchial lung biopsy (6-9). BAL is recommended by current guidelines for patients with newly detected ILD of apparently unknown cause and an unclear radiological pattern, although the quality of evidence is considered very low (5,10).

The 2012 American Thoracic Society clinical practice guidelines recommend that BAL be classified according to the predominance of specific cell types (7). Various ILD subtypes are considered to exhibit characteristic cytological features that can be identified in BAL, such as lymphocyte predominance in hypersensitivity pneumonitis (HP) and sarcoidosis, and eosinophilic predominance in chronic eosinophilic pneumonia (CEP) (4,11-13). However, there is considerable overlap between different ILD, limiting the diagnostic utility of BAL (14).

In recent years, ILD have been classified based on the presence or absence of fibrosis, and there is sparse data comparing the utility of BAL in fibrotic vs non-fibrotic ILD. For example, the 2020 ATS/JRS/ALAT guidelines recommend that patients with HP be classified as having fibrotic HP or non-fibrotic HP, rather than using the historic categories of acute, subacute, and chronic (4). However,

most prior studies assessed the diagnostic value of BAL in HP using the older definition of chronic HP (15). A recent Canadian study investigated BAL results in fibrotic ILDs, showing a similar low frequency of BAL lymphocytosis in fibrotic HP and in UIP, suggesting that BAL cell counts may lack the ability to differentiate these two clinically important entities (16). However, the authors did not include patients with non-fibrotic diseases. This missing piece of the puzzle is important for shaping the clinical indications for BAL in fibrotic and non-fibrotic ILD.

Considering the above, the objective of this multicenter real-life study was to evaluate the differences in BAL cellular profiles and their radiological correlates across the spectrum of fibrotic and non-fibrotic ILDs.

Materials and Methods

Study design and participants

This was a multicenter observational study utilizing three retrospective ILD registries from tertiary medical centers in Israel. Registries included consecutive patients with ILD who were followed in the ILD clinic at each center. In the current study, our cohort included all adult patients (>18 years) who underwent bronchoscopy between 2017 and 2022 (Figure 1). The participating centers were Tel Aviv Sourasky Medical Center, Carmel Medical Center, and Meir Medical Center. The study was approved by the Tel Aviv Sourasky Medical Center review board (21-0456-TLV), without the need for explicit consent from the participants due to its retrospective design. The study is reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations (17).

Bronchoscopic evaluations were identified by screening patients' electronic medical records (pulmonologist visits, hospitalizations, and other related clinic visits), as described in details before (18). To be eligible for study inclusion, patients had to have an ILD diagnosis based on a multidisciplinary discussion (MDD) in each medical center according to accepted standards (19). In short, each MDD included a pulmonologist specialized in ILD, a chest radiologist, a

pathologist specialized in lung histology, and, in relevant cases, rheumatologists. A diagnosis was determined by the MDD for confident or provisional confidence diagnoses (according to Ryerson et al. (1)). In cases of lower confidence (i.e., <50%), it was determined as “unclassifiable”. Fibrotic ILD were categorized as IPF, idiopathic non-specific interstitial pneumonia (iNSIP), fibrotic HP, connective tissue-related interstitial lung disease (CTD-ILD), and sarcoidosis. Non-fibrotic subtypes were categorized as non-fibrotic HP, organizing pneumonia (OP), exposure-related (e.g., RB-ILD, occupational), CTD-ILD, sarcoidosis, and eosinophilic pneumonia (EP).

In general, evaluation of patients with ILD in the study's centers includes an assessment of pulmonary and extra-pulmonary symptoms, occupational and environmental exposures, physical examination, and analysis of autoantibodies (minimum of ANA, RF, and anti-CCP). In cases of NSIP radiologic pattern, unexplained ILD, or suspicion of CTD-ILD, a more extended panel is ordered, including SLC-70, anti-centromere, anti-Ro, anti-I.a, anti-RNP, anti-dsDNA, etc. Myositis panel (MDA5, PM-SCL, Jo-1, PL-7, PL-12, EJ, OJ) is ordered on a case-by-case basis. Rheumatologic consultation is ordered when CTD is suspected, for example, in patients with physical signs, positive autoantibodies, or NSIP radiologic pattern. Trans-bronchial biopsies are indicated for cases with uncertainty in diagnosis, for example, cases of indeterminate UIP or UIP pattern with an HP-related exposure. Cryobiopsy is preferred for fibrotic ILD, although comorbidities and procedural risk are taken into account on a personalized basis.

All chest high-resolution CT (HRCT) scans were analyzed for the purpose of the study by a radiologist specialized in chest imaging who was blinded to clinical data. The radiologic assessment determined whether the ILD was fibrotic or non-fibrotic and whether there was evidence of ground glass opacities and mosaic attenuation (20). ILD was defined as fibrotic based on the presence of reticulation, traction bronchiectasis, and/or honeycombing. In addition, all cases were classified for the presence of a UIP pattern, based on accepted guidelines, with definite, probable, and indeterminate UIP patterns counted as UIP for comparison purposes (5).

Procedures

Bronchoscopy, BAL, and trans-bronchial biopsies were performed according to accepted practice (2,7,19) and as part of the initial evaluation of patients with ILD. Bronchoscopies included in this study were not performed during an ILD exacerbation in all cases of IPF, CTD-ILD, HP, iNSIP, smoking-related ILD, or sarcoidosis. Considering the nature of OP, EP, and drug-associated ILD, bronchoscopies were mostly performed in a more acute setting in proximity to symptomatic worsening, as often done in clinical practice. All anticoagulants were discontinued before the procedure according to guidelines. Patients received oxygen throughout the procedure and were continuously monitored. Moderate or deep sedation was administered by increasing doses of intravenous midazolam, fentanyl, and/or propofol by the anesthetist or anesthesiologist. The target site for all BAL procedures was based on the most adjacent chest HRCT findings. As recommended by the American Thoracic Society Clinical Practice Guideline, areas of alveolar ground glass opacity, more prominent nodular profusion, or fine reticulation were prioritized for BAL collection (7). BAL was performed by wedging the bronchoscope in a lobar or sub-lobar bronchus and instilling 120-180 ml of warmed saline (37°C). BAL fluid was considered adequate if more than 30% of the instilled volume was retrieved.

For cell analysis, BAL was filtered to remove mucus, then sediment was made from cellular material by centrifugation (292 g for 10 min at room temperature) and expanded in RPMI-1640 containing 10% Male AB human serum, 1% Penicillin 10 KU - Streptomycin 10mg/mL and 1% L-Glutamine Solution, 200mM. Differential cell count of 500 BAL cells was performed on May-Grunwald-Giemsa stained preparations (7). In this work, we used specific features of BAL cell counts, based on the American Thoracic Society Clinical Practice Guideline on BAL and prior works related to this topic (1,7). A mixed pattern was defined as two or three of the following: neutrophils >3%, eosinophils >1%, and lymphocytes >15%. The sole appearance of one of these three features was also analyzed. We also chose to address the following features that were previously found to be associated with severity or diagnosis

of ILD: neutrophils >4.5%, lymphocytes >20%, lymphocytes >30%, and CD4/8 ratio >0.8 (21).

Study outcomes

Our primary outcome was BAL differential indices in fibrotic vs non-fibrotic ILD, with cutoffs based on prior works, as mentioned above. Our secondary outcomes included BAL differential results in specific fibrotic and non-fibrotic ILDs, their predictive utility for fibrotic HP diagnosis (with emphasis on BAL lymphocyte counts), and their correlations with specific radiologic features.

Statistical analysis

Data were analyzed for the entire cohort and separately for fibrotic and non-fibrotic ILDs. Additional sub-analyses were made with a focus on fibrotic ILDs, mainly on CTD-ILD, IPF, and fibrotic HP. The distribution of continuous data was assessed by the Kolmogorov-Smirnov test. Continuous variables with non-normal distribution were presented as median (interquartile range, IQR) and compared between groups with Mann-Whitney U tests. Continuous variables with normal distribution were presented as mean \pm standard deviation (SD) and compared between groups with t-tests. Categorical variables were presented as sum (percentage of total) and compared by Chi-square tests or Fisher's exact test when appropriate. Given that BAL lymphocyte count had significant associations with non-fibrotic disease and with HP diagnosis, we performed a multivariate linear regression analysis using lymphocyte counts as a continuous variable. We included variables that correlated with BAL lymphocyte counts, and with age, gender, and smoking status as predefined variables considering their associations with ILD subtype and the association between smoking and BAL counts (22). There was no missing data. Analyses were performed in SPSS version 29.0, with $p < 0.05$ as the threshold for statistical significance.

Results

In total, 238 patients underwent bronchoscopy with BAL as part of their ILD diagnostic workup and were included in this study (Figure 1). The cohort's mean age was 60 years, and 46% were female. Overall, 120 (50.4%) patients were diagnosed with fibrotic ILD and 118 (49.6%) with non-fibrotic ILD, as presented

in Table 1. A list of all MDD diagnoses appears in the supplementary results. Of patients with fibrotic ILD, the HRCT pattern was UIP in 45 cases (38%), including 12 indeterminate UIP, 15 probable UIP, and 18 definite UIP patterns. Ninety-one percent of the cohort completed trans-bronchial biopsies (forceps or cryo) during their bronchoscopy. Cryobiopsy was performed in 75% of iNSIP diagnoses, 66% of fibrotic HP diagnoses, and 86% of IPF diagnoses.

BAL differential in fibrotic vs non-fibrotic ILD

Comparison of BAL differential between fibrotic and non-fibrotic ILD is shown in Table 2. The non-fibrotic group had a higher frequency of >20% lymphocytes (36.4% vs. 17.5%, $P<0.001$) and >30% lymphocytes (25.4% vs. 9.2%, $P<0.001$). There was a non-significant trend towards more mixed patterns in fibrotic ILD (71% vs. 60%, $p=0.08$). There were no significant differences between the fibrotic and non-fibrotic groups in other indices. The findings were similar in a subgroup analysis excluding patients with sarcoidosis and eosinophilic pneumonia (supplementary Table S1). In a multivariate linear regression model, there was a negative association between fibrotic ILD and BAL lymphocyte counts (supplementary Table S2, $\beta=-6.15$, $p=0.007$), while the diagnosis of HP was an independent predictor for higher lymphocyte counts ($\beta=6.91$, $p=0.010$). Interestingly, for all ILDs that may present as both fibrotic or non-fibrotic, BAL lymphocytosis >20% was more frequent in the non-fibrotic form, including in HP (60 vs. 21%, $p=0.005$), CTD-ILD (33% vs. 11%, $p=0.14$), and sarcoidosis (46% vs. 17%, $p=0.07$).

BAL differential in specific fibrotic and non-fibrotic ILD

BAL differential results in patients with fibrotic ILD are presented in Table 3. Among patients with fibrotic ILD, the mixed BAL pattern was highest in IPF (86%) and fibrotic HP (79%), while neutrophils above 3% as a sole finding were highest in iNSIP (44%) and CTD-ILD (28%). Although rates of lymphocytes above 20% were similar between most ILD diagnoses, results above 30% were more prevalent among fibrotic HP patients, while still occurring in 21% of patients. Importantly, the median percentage of lymphocytes was not different between the various fibrotic ILD etiologies.

In non-fibrotic ILDs (Table 4), lymphocytes >20% were most prevalent in non-fibrotic HP (60% vs. 13-46% in others), while counts above 30% were comparable in HP (40%), OP (37%), and sarcoidosis (29%). The mixed pattern was lowest in patients with exposure-related ILD (41%), which had the highest rate of neutrophil count above 3% as the sole BAL finding (55%). The median percentage of eosinophils was low in all non-fibrotic entities, except for eosinophilic pneumonia (49% vs. 0.55-2.3%) (Table 4).

BAL differential between fibrotic HP and other ILD

Considering the focus given to BAL in the diagnostic workup of HP (4), especially among fibrotic ILD, which often conveys a more difficult diagnostic question, we assessed the differences in BAL in this subpopulation (Table 5). Patients with fibrotic HP, compared to other fibrotic diseases, had a lower rate of isolated neutrophils >3% (3% vs. 26%, $p=0.008$) and a higher rate of lymphocytes >30% (21% vs. 6%, $p=0.023$). In a sub-group analysis (supplementary Table S3), the higher rates of lymphocytes >30% in fibrotic HP patients persisted only when compared to those with a UIP pattern by HRCT, while they were not different compared to those with a non-UIP pattern or with sarcoidosis. Patients with fibrotic HP had higher rates of lymphocytes >30% than those with IPF (21% vs. 0%, $p=0.033$), with all other indices similar between the groups. Sensitivity analyses of different BAL thresholds to predict fibrotic HP diagnosis among all fibrotic ILDs and only compared to IPF are presented in Supplementary Table S4. Across all fibrotic ILDs, a 30% lymphocytes cutoff for HP diagnosis had a specificity of 95% and NPV of 79% with a low PPV of 55%. In comparison, among only IPF and fibrotic HP patients, this cutoff had a specificity of 100%, a low NPV of 48%, and a 100% PPV.

Sub-group analyses and correlations with radiologic features

In a sub-group analysis of fibrotic ILD without sarcoidosis, patients with honeycombing ($n=21$) had a lower rate of neutrophils >4.5% in their BAL (71% vs. 91%, $p=0.018$), while all other indices were similar (supplementary Table S5). An additional sub-analysis was performed to evaluate the association between BAL lymphocytosis and imaging features, also after excluding patients with sarcoidosis, given their distinct features. Rates of BAL lymphocytes >20%

or >30% were similar regardless of the presence or absence of GGOs or mosaic attenuation, in both the fibrotic and non-fibrotic groups (Table 6). Finally, in a sub-analysis based on smoking status (supplementary Table S6), there were no differences between groups in terms of diagnoses or BAL differential results.

Discussion

This study is, to our knowledge, the first multicenter analysis to compare BAL cellular profiles across the full spectrum of ILD by fibrosis status, with multidisciplinary diagnoses as the reference. Prior work either used old definitions (e.g., chronic HP) (15) or evaluated BAL in fibrotic ILD only (16), without a comparison to non-fibrotic ILD. In 238 consecutively evaluated patients, we show that BAL lymphocytosis (>20% and >30%) is consistently more frequent in non-fibrotic vs fibrotic ILD, and that for diagnoses with both phenotypes (HP, CTD-ILD, sarcoidosis), non-fibrotic forms show higher lymphocyte yields. We also show no association between lymphocytosis and GGO or mosaic attenuation in either fibrosis stratum, extending prior observations limited to fibrotic ILD (16). Our real-world findings suggest that BAL has distinctive cellular patterns in non-fibrotic ILD, while it seems to have a limited utility as a stand-alone test in fibrotic ILD.

Several ILD are known to have distinctive features in their cellular BAL profile. Sarcoidosis typically shows lymphocytic predominance, and the CD4/CD8 ratio is widely used as part of the basic BAL evaluation in suspected cases (23). Still, lymphocyte subset analysis is not recommended as a routine component of BAL analysis, given its relatively low sensitivity and variability during the disease course (7). Eosinophilic predominance in BAL is considered diagnostic for conditions such as chronic eosinophilic pneumonia (CEP) or other eosinophilic ILDs, particularly when considered in the appropriate clinico-radiologic context (24). The mentioned findings are consistent with our results. When these specific diagnoses are considered, BAL might offer information that helps guide MDD decisions in narrowing down potential causes of ILD or to support a leading diagnosis.

In contrast, we have found that fibrotic ILD as a group does not exhibit such clear cellular patterns on BAL as non-fibrotic ILD. This finding may be attributed

to the advanced fibrotic changes, which could mask any early or subtle inflammatory features that might otherwise be observed in the BAL analysis. As fibrosis progresses, the pathologic changes in the lung tissue may become more structural and less responsive to the types of inflammatory processes, especially lymphocytosis, that are often detected through BAL in non-fibrotic ILDs (25). Based on our sensitivity analysis, lymphocyte counts above 30% had an association with HP among fibrotic ILDs, and this association changes based on the differential diagnosis. In specific cases when the differential is between fibrotic HP and IPF, meeting this cutoff results in 100% specificity and positive predictive value, although this cutoff was present in only 21% of patients with fibrotic HP in our cohort, resulting in a very low sensitivity.

Our results align with previous studies that found no distinct cellular features in fibrotic ILD. A recently published study by Grant-Orser et al. (16) evaluated 209 cases for the association of BAL with radiologic features, patterns, and clinical diagnoses. The study categorized BAL using guideline-recommended thresholds, similar to those presented in our work. The investigators found that BAL lymphocytosis occurred with similar frequency across HRCT patterns of fibrotic hypersensitivity pneumonitis (21%) and usual interstitial pneumonia (18%). Interestingly, only 5% of patients with MDD-based fibrotic hypersensitivity pneumonitis had isolated lymphocytosis > 15%. The study concluded that BAL cellular analyses did not significantly correlate with radiologic features, guideline patterns, or MDD-based diagnoses, which is consistent with our findings regarding the limited value of BAL in fibrotic ILDs.

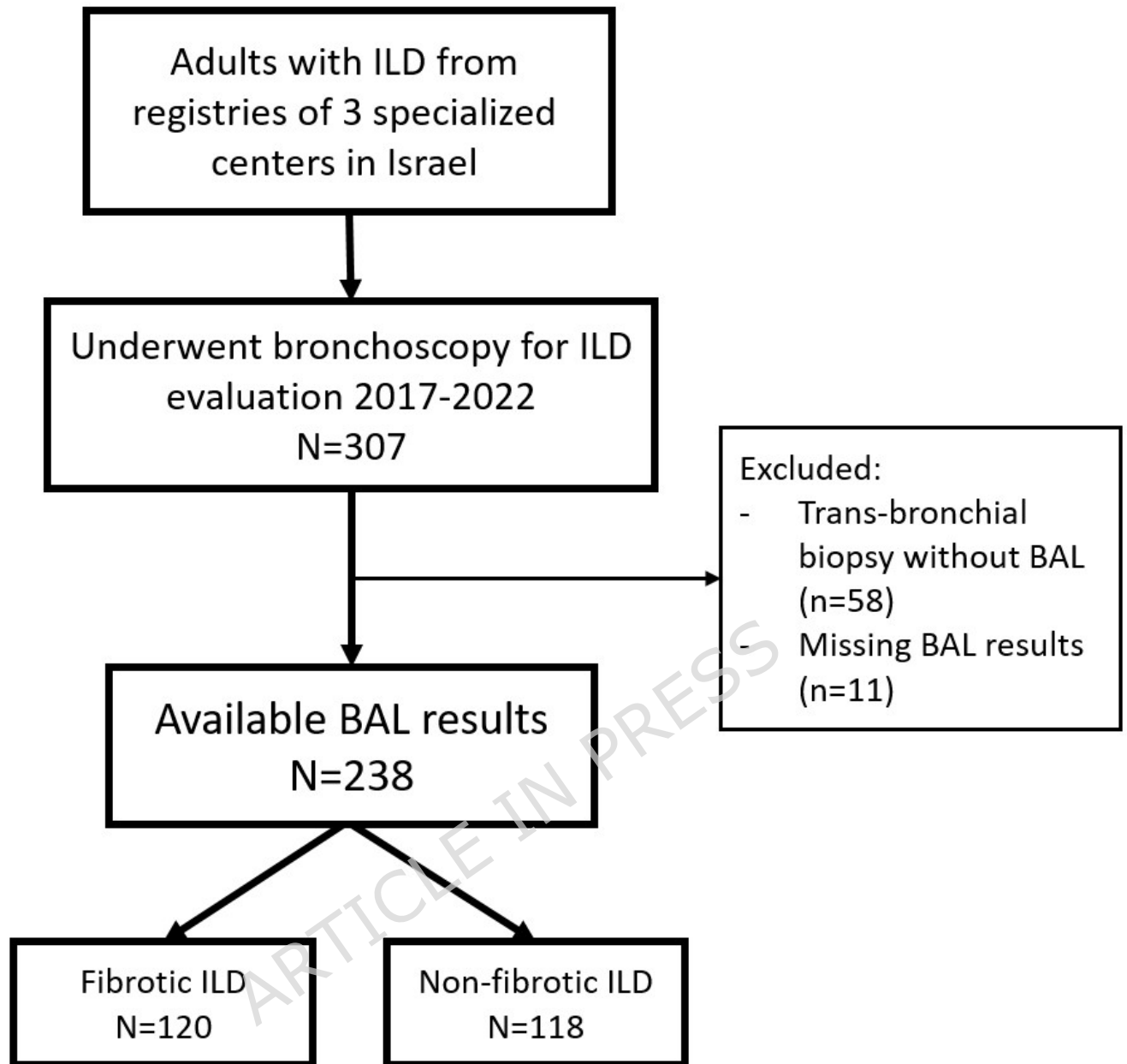
HP is one of the only ILD in which BAL results have been included in the official diagnostic criteria. Based on the ATS/JRS/ALAT Clinical Practice Guideline for HP diagnosis, patients with a typical HP pattern in HRCT, relevant exposure, and BAL lymphocytosis do not require additional testing (4). One might assume that bronchoscopy should first be performed only for BAL, and in cases when uncertainty remains, a second bronchoscopy should be performed with trans-bronchial biopsy. Our results suggest against this strategy, especially in fibrotic disease. Even though rates of lymphocyte count above 30% were higher in

fibrotic HP compared to other fibrotic ILD or to IPF, this was present in only 21% of the fibrotic HP cases, resulting in a low diagnostic yield.

This study has several limitations. First, its retrospective design, which may introduce selection bias, as patients were sent to BAL only if clinically indicated. With that said, we believe that our consecutive multicenter study population is a reliable real-life representation. Second, the lack of long-term follow-up data, which would have provided insight into the outcomes and progression of ILD subtypes over time, limits the assessment of BAL prognostic yield. Third, while the study examined a diverse cohort of patients, the sample size for certain ILD subtypes, such as sarcoidosis and eosinophilic pneumonia, was relatively small, potentially limiting the generalizability of the findings. Fourth, the heterogeneity of the CTD-ILD group—which we treated as a uniform subgroup due to the small number of patients—may have diluted disease-specific BAL signatures. Fifth, as MDDs were made after the BAL results and were assessed retrospectively, we cannot ascertain the impact of BAL on the final MDD diagnosis, leading to incorporation bias. This is especially relevant for HP diagnosis, given that in our clinical practice, only BAL lymphocytosis (or its absence) is truly considered in MDD discussions to support HP diagnosis, hence impacting its identified association with BAL lymphocytosis. We still believe that this limitation is minimized by the high rate of trans-bronchial biopsies (higher diagnostic confidence) and the in-depth evaluations made in the three ILD centers. Finally, considering the mentioned limitation and a prior work on this topic, assessment of trans-bronchial biopsy results was beyond the study scope (18).

In conclusion, while BAL frequently demonstrated cellular differences in non-fibrotic ILDs, it infrequently provided distinctive features in fibrotic ILDs. Thus, the utility of BAL as a stand-alone diagnostic modality in fibrotic ILD workup seems to be limited. Further prospective studies with larger, more diverse cohorts and long-term follow-up are needed to refine the role of BAL in the diagnostic evaluation and management of fibrotic ILDs.

Figure 1, Study inclusion process.



Abbreviations: BAL, bronchoalveolar lavage; ILD, interstitial lung disease.

Declarations:

Ethics approval and consent to participate: The study was approved by the Tel Aviv Sourasky Medical Center review board (21-0456-TLV) and conducted per the declaration of Helsinki and GCP Guidelines. Informed consent was waived given the retrospective nature of our study.

Consent for publication: Not applicable.

Author contributions: E.G. and A.U. conceived and designed the study. O.F., J.B., E.K., D.C.S., Y.A., Y.H., G.A., D.S., and T.H. performed data acquisition. E.G., O.F., O.W., S.S., A.B.S., and A.U. performed data analysis and interpretation. E.G., O.F. and A.U. drafted the manuscript. A.U. supervised the project. All authors critically revised the manuscript and approved the final version.

Acknowledgment: none.

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

Conflict of Interest statement: A.U. reports receiving research funding from Boehringer Ingelheim and the Pulmonary Fibrosis Foundation, personal consulting fees from Boehringer Ingelheim, RemedyCell, Splisense, Augmanity Nano, and 1E Therapeutics in the last 36 months, all outside the submitted work. Y.A. reports personal fees from Teva, grants and personal fees from GSK and AstraZeneca, and personal fees from Sanofi, BI and Kamada, outside the submitted work. A.B.S. reports receiving personal consulting fees and lecture fees from Sanofi-Regeneron, Astrazeneca, GSK, Kamada, Boehringer Ingelheim, Roche, outside the submitted work. All other authors report no conflict of interest.

Availability of data and materials: All relevant data and analyses are given within the manuscript.

References

1. Ryerson CJ, Corte TJ, Lee JS, Richeldi L, Walsh SLF, Myers JL, et al. A Standardized Diagnostic Ontology for Fibrotic Interstitial Lung Disease. An International Working Group Perspective. *Am J Respir Crit Care Med*. 2017 Nov 15;196(10):1249-54. doi:10.1164/rccm.201702-0400PP
2. Colella S, Haentschel M, Shah P, Poletti V, Hetzel J. Transbronchial Lung Cryobiopsy in Interstitial Lung Diseases: Best Practice. *Respiration*. 2018 Jun 12;95(6):383-91. doi:10.1159/000488910
3. Tomassetti S, Piciucchi S, Tantalocco P, Dubini A, Poletti V. The multidisciplinary approach in the diagnosis of idiopathic pulmonary fibrosis: a patient case-based review. *Eur Respir Rev*. 2015 Feb 28;24(135):69-77. doi:10.1183/09059180.00011714
4. Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vasakova M, et al. Diagnosis of Hypersensitivity Pneumonitis in Adults: An Official ATS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2020 Aug;202(3):e36-69. doi:10.1164/rccm.202005-2032ST
5. Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, 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*. 2022 May;205(9):e18-47. doi:10.1164/rccm.202202-0399ST
6. Grutters JC. Establishing a Diagnosis of Pulmonary Sarcoidosis. *J Clin Med*. 2023 Jan;12(21):21. doi:10.3390/jcm12216898
7. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al. An Official American Thoracic Society Clinical Practice Guideline: The Clinical Utility of Bronchoalveolar Lavage Cellular Analysis in Interstitial Lung Disease. *Am J Respir Crit Care Med*. 2012 May;185(9):1004-14. doi:10.1164/rccm.201202-0320ST
8. Adams TN, Newton CA, Batra K, Abu-Hijleh M, Barbera T, Torrealba J, et al. Utility of Bronchoalveolar Lavage and Transbronchial Biopsy in Patients with Hypersensitivity Pneumonitis. *Lung*. 2018 Oct 1;196(5):617-22. doi:10.1007/s00408-018-0139-1
9. Pérez ERF, Travis WD, Lynch DA, Brown KK, Johannson KA, Selman M, et al. Diagnosis and Evaluation of Hypersensitivity Pneumonitis: CHEST Guideline and Expert Panel Report. *CHEST*. 2021 Aug 1;160(2):e97-156. doi:10.1016/j.chest.2021.03.066 PubMed PMID: 33861992.
10. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*. 2018 Sep;198(5):e44-68. doi:10.1164/rccm.201807-1255ST

11. Suzuki Y, Suda T. Eosinophilic pneumonia: A review of the previous literature, causes, diagnosis, and management. *Allergol Int.* 2019 Oct 1;68(4):413–9. doi:10.1016/j.alit.2019.05.006
12. Cottin V. Eosinophilic Lung Diseases. *Clin Chest Med.* 2016 Sep 1;Rare and Orphan Lung Diseases37(3):535–56. doi:10.1016/j.ccm.2016.04.015
13. Freund O, Hadad Y, Shalmon T, Wand O, Schneer S, Perluk TM, et al. Real-Life Diagnostic Performance of the Hypersensitivity Pneumonitis Guidelines: A Multicenter Cohort Study. *Diagnostics.* 2023 Jan;13(14):14. doi:10.3390/diagnostics13142335
14. Efared B, Ebang-Atsame G, Rabiou S, Diarra AS, Tahiri L, Hammas N, et al. The diagnostic value of the bronchoalveolar lavage in interstitial lung diseases. *J Negat Results Biomed.* 2017 Mar 1;16(1):4. doi:10.1186/s12952-017-0069-0
15. Adderley N, Humphreys CJ, Barnes H, Ley B, Premji ZA, Johannson KA. Bronchoalveolar lavage fluid lymphocytosis in chronic hypersensitivity pneumonitis: a systematic review and meta-analysis. *Eur Respir J.* 2020 Aug;56(2):2000206. doi:10.1183/13993003.00206-2020 PubMed PMID: 32265306.
16. Grant-Orser A, Asmussen M, Marinescu DC, Hague CJ, Muller NL, Murphy DT, et al. BAL Fluid Cellular Analysis and Radiologic Patterns in Patients With Fibrotic Interstitial Lung Disease. *CHEST.* 2025 Jan 1;167(1):172–82. doi:10.1016/j.chest.2024.07.166 PubMed PMID: 39179174.
17. Lachat C, Hawwash D, Ocke MC, Berg C, Forsum E, Hörnell A, et al. Strengthening the Reporting of Observational Studies in Epidemiology—Nutritional Epidemiology (STROBE-nut): An Extension of the STROBE Statement. *PLOS Med.* 2016;13(6):e1002036. doi:10.1371/journal.pmed.1002036
18. Freund O, Wand O, Schneer S, Barel N, Shalmon T, Borsekofsky S, et al. Transbronchial Cryobiopsy Is Superior to Forceps Biopsy for Diagnosing both Fibrotic and Non-Fibrotic Interstitial Lung Diseases. *Respiration.* 2023 Aug 25;102(9):852–60. doi:10.1159/000533197
19. Bradley B, Branley HM, Egan JJ, Greaves MS, Hansell DM, Harrison NK, et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. *Thorax.* 2008 Sep;63 Suppl 5:v1-58. doi:10.1136/thx.2008.101691 PubMed PMID: 18757459.
20. Shalmon T, Freund O, Wand O, Schneer S, Hershko T, Hadad Y, et al. Hypersensitivity pneumonitis radiologic features in interstitial lung diseases. *Respir Med.* 2025 Jan 1;236. doi:10.1016/j.rmed.2024.107901 PubMed PMID: 39631548.

21. Frye BC, Schupp JC, Rothe ME, Köhler TC, Prasse A, Zissel G, et al. The value of bronchoalveolar lavage for discrimination between healthy and diseased individuals. *J Intern Med*. 2020;287(1):54–65. doi:10.1111/joim.12973
22. Karimi R, Tornling G, Grunewald J, Eklund A, Sköld CM. Cell Recovery in Bronchoalveolar Lavage Fluid in Smokers Is Dependent on Cumulative Smoking History. *PLOS ONE*. 2012;7(3):e34232. doi:10.1371/journal.pone.0034232
23. Rossides M, Darlington P, Kullberg S, Arkema EV. Sarcoidosis: Epidemiology and clinical insights. *J Intern Med*. 2023;293(6):668–80. doi:10.1111/joim.13629
24. Campos LEM, Pereira LFF. Eosinofilia pulmonar. *J Bras Pneumol*. 2009 Jun;35:561–73. doi:https://doi.org/10.1590/S1806-37132009000600010
25. Barnett JL, Maher TM, Quint JK, Adamson A, Wu Z, Smith DJF, et al. Combination of BAL and Computed Tomography Differentiates Progressive and Non-progressive Fibrotic Lung Diseases. *Am J Respir Crit Care Med*. 2023 Nov 1;208(9):975–82. doi:10.1164/rccm.202305-0796OC

Table 1, Cohort characteristics.

Variable	Total n=238 (%)	Non fibrotic n=118 (%)	Fibrotic n=120 (%)	p
Age, years, mean \pm SD	60 \pm 13	59 \pm 14	64 \pm 12	<0.001
Female sex	110 (46)	62 (53)	48 (40)	0.054
Charlson score, median (IQR)	3 (2-4)	2 (1-3)	3 (2-4)	0.004
Current or former smokers	105 (44)	45 (39)	60 (50)	0.230
Additional lung disease ^a	26 (11)	9 (8)	17 (14)	0.106
Underwent TBB	217 (91)	108 (91)	109 (91)	0.854
Lung functions (n=215):				
FEV1% - predicted, mean \pm SD	78 \pm 18	77 \pm 21	78 \pm 19	0.790
FVC% - predicted, mean \pm SD	79 \pm 17	81 \pm 20	77 \pm 18	0.113
FEV1/FVC, mean \pm SD	0.74 \pm 0.15	0.71 \pm 0.17	0.76 \pm 0.19	0.843
TLC% - predicted, mean \pm SD	83 \pm 20	88 \pm 21	80 \pm 20	<0.001
DLCOc% - predicted, mean \pm SD	57 \pm 19	60 \pm 19	56 \pm 18	0.236

Abbreviations: DLCOc, diffusing capacity of the lungs for carbon monoxide, corrected for hemoglobin; FEV1, Forced Expiratory Volume in 1 second; FVC, forced vital capacity; TBB, trans-bronchial biopsy; TLC, total lung capacity.

^a Including asthma and COPD.

Table 2, Bronchoalveolar lavage results in the study cohort, compared between fibrotic and non-fibrotic disease.

Variable	Total N=238 (%)	Non fibrotic n=118 (%)	Fibrotic n=120 (%)	p
Only lymphocytes >15%	13 (6)	8 (7)	5 (4)	0.408
Only eosinophils >1%	4 (2)	3 (3)	1 (1)	0.368
Only neutrophils >3%	59 (25)	34 (29)	25 (21)	0.154
Mixed pattern	156 (66)	71 (60)	85 (71)	0.083
Neutrophils >4.5%	189 (79)	89 (75)	100 (83)	0.131
Lymphocytes >20%	64 (27)	43 (36)	21 (18)	<.001
Lymphocytes >30%	41 (17)	30 (25)	11 (9)	<.001
CD4/CD8 ratio >0.8*	125 (71)	61 (66)	64 (78)	0.071
CD4/CD8 ratio >3.5*	31 (18)	15 (16)	16 (20)	0.559

* Data available for 175 (74%) patients.

Table 3, Bronchoalveolar lavage results in different fibrotic interstitial lung diseases

Variable	IPF n=21 (%)	HP n=29 (%)	CTD- ILD n=18 (%)	iNSIP n=16 (%)	Sarcoidosis n=12 (%)	Others n=24 (%)
Macrophages, %	46 (31-69)	61 (32-73)	55 (28-66)	54 (20-75)	72 (44-86)	29 (15-64)
Neutrophils, %	28 (8-66)	17 (6-25)	32 (9-62)	22 (12-54)	5 (2-11)	37 (16-72)
Lymphocytes, %	8 (6-11)	13 (10-18)	11 (8-15)	10 (5-17)	11 (7-17)	8 (3-19)
Eosinophils, %	4.6 (2-9.6)	3 (2-7)	1.5 (0.2-6)	1 (0-3)	0.9 (0.2-3)	2 (0.8-7.1)
CD4/CD8 ^a	1.6 (0.8-3.5)	2.1 (1.4-3.2)	0.9 (0.6-1.4)	1.6 (1.3-7.7)	2.1 (1.2-2.8)	1.5 (0.9-2.1)
Only lymphocytes >15%	0 (0)	3 (10)	1 (6)	0 (0)	1 (8)	0 (0)
Only eosinophils >1%	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Only neutrophils >3%	3 (14)	1 (3)**	5 (28)	7 (44)*	3 (25)	6 (25)
Mixed pattern	18 (86)	23 (79)	12 (67)	9 (56)	5 (42)	18 (75)
Neutrophils >4.5%	18 (86)	23 (79)	16 (89)	15 (94)	6 (50)**	22 (92)
Lymphocytes >20%	2 (10)	6 (21)	2 (11)	3 (19)	2 (17)	6 (25)
Lymphocytes >30%	0 (0)	6 (21)*	1 (6)	2 (13)	1 (8)	1 (4)
CD4/CD8 ratio >0.8 ^a	9 (75)	18 (82)	5 (50)	6 (86)	11 (92)	15 (79)

Abbreviations: CTD-ILD, connective-tissue disease-related interstitial lung disease; HP, hypersensitivity pneumonitis; iNSIP, idiopathic non-specific interstitial pneumonia; IPF, idiopathic pulmonary fibrosis.

^a Data available for 82 patients.

* p<0.05, ** p<0.01

Table 4, Bronchoalveolar lavage results in different non-fibrotic interstitial lung diseases

Variable	HP n=20 (%)	OP n=19 (%)	Exposu re ^a n=22 (%)	CTD- ILD n=12 (%)	Sarcoid osis n=24 (%)	EP n=13 (%)	Others n=8 (%)
Macrophages, %	24 (11-55)	36 (15-54)	57 (37-74)	49 (19-65)	55 (30-72)	17 (7-33)	65 (22-84)
Neutrophils, %	24 (5-61)	28 (13-46)	16 (8-47)	16 (10-48)	13 (2-43)	4 (3-21)	14 (3-63)
Lymphocytes, %	26 (9-44)	14 (7-44)	8 (5-14)	10 (4-25)	17 (8-33)	5 (0.8-20)	10 (2-15)
Eosinophils, %	1.8 (0.5-3.2)	1.8 (0-10)	0.8 (0-1.2)	2.3 (0.1-3.1)	0.55 (0.2-1.6)	49 (26-69)	0.9 (0-3.2)
CD4/CD8 ^a	1 (0.8-3.7)	0.5 (0.3-1.6)	0.9 (0.6-1.6)	1.3 (0.8-1.6)	1.8 (1.2-4.3)	1.2 (0.8-1.9)	1 (0.4-1.8)
Only lymphocytes >15%	1 (5)	1 (5)	1 (5)	0 (0)	4 (17)	0 (0)	1 (13)
Only eosinophils >1%	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (23)**	0 (0)
Only neutrophils >3%	4 (20)	3 (16)	12 (55)**	4 (33)	8 (33)	1 (8)	2 (25)
Mixed pattern	15 (75)	15 (79)	9 (41)	8 (67)	11 (46)	9 (69)	4 (50)
Neutrophils >4.5%	15 (75)	17 (90)	19 (86)	11 (92)	16 (67)	6 (46)*	5 (63)
Lymphocytes >20%	12 (60)*	8 (42)	4 (18)	4 (33)	11 (46)	3 (23)	1 (13)
Lymphocytes >30%	8 (40)	7 (37)	2 (9)	3 (25)	7 (29)	2 (15)	1 (13)
CD4/8 ratio >0.8 ^b	10 (63)	5 (45)	10 (59)	4 (67)	20 (83)*	8 (72)	4 (50)

Abbreviations: CTD-ILD, connective-tissue disease-related interstitial lung disease; EP, eosinophilic pneumonia; HP, hypersensitivity pneumonitis; OP, organizing pneumonia.

^a Includes environmental and occupational exposures, excluding hypersensitivity pneumonitis.

^b Data available for 93 patients.

* p<0.05, ** p<0.01

Table 2, Comparison of bronchoalveolar lavage results between fibrotic HP and other fibrotic interstitial lung diseases.

Variable	Fibrotic HP n=29 (%)	non-HP fibrotic ILD n=91 (%)	IPF n=21 (%)	p1^a	p2^a
Only lymphocytes >15%	3 (10)	2 (2)	0 (0)	0.090	0.254
Only neutrophils >3%	1 (3)	24 (26)	3 (14)	0.008	0.297
Mixed pattern	23 (79)	62 (68)	18 (86)	0.249	0.716
Neutrophils >4.5%	23 (79)	77 (85)	18 (86)	0.504	0.716
Lymphocytes >20%	6 (21)	15 (17)	2 (10)	0.585	0.441
Lymphocytes >30%	6 (21)	5 (6)	0 (0)	0.023	0.033
CD4/8 ratio >0.8	18 (62)	47 (52)	10 (48)	0.327	0.310

Abbreviations: HP, hypersensitivity pneumonitis; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis.

^a p1 - comparison between fibrotic HP and non-HP fibrotic ILD; p2 - comparison between fibrotic HP and IPF.

Table 3, Associations between radiologic features and bronchoalveolar lavage lymphocytes count *.

Variable	GGO n=121 (%)	No GGO n=81 (%)	p	Mosaic n=50 (%)	No mosaic n=152 (%)	p
Lymphocytes %	11.0 (6- 23)	11.0 (5- 20)	0.643	10.6 (7- 16)	11.0 (5- 21)	0.881
Lymphocytes >20%	31 (26)	20 (25)	0.882	11 (22)	40 (26)	0.542
Lymphocytes >30%	22 (18)	11 (14)	0.386	8 (16)	25 (16)	0.941
Fibrotic interstitial lung disease (n=108)						
Lymphocytes %	10.6 (7- 14)	11.2 (6- 18)	0.637	10.2 (7- 12)	11.0 (6- 17)	0.315
Lymphocytes >20%	9 (15)	10 (21)	0.377	2 (8)	17 (21)	0.148
Lymphocytes >30%	6 (10)	4 (9)	0.814	2 (8)	8 (10)	1.000
Non-fibrotic interstitial lung disease (n=94)						
Lymphocytes %	13.2 (5- 31)	10.7 (2- 25)	0.333	11.8 (6- 30)	11.6 (4- 30)	0.396
Lymphocytes >20%	22 (37)	10 (29)	0.476	9 (39)	23 (32)	0.554
Lymphocytes >30%	16 (27)	7 (21)	0.51	6 (26)	17 (24)	0.835

Abbreviations: GGO, ground glass opacities.

* Patients with sarcoidosis were excluded from this analysis, given their distinct features.

Muster der Bronchoalveolärlavage bei fibrotischen und nicht-fibrotischen interstitiellen Lungenerkrankungen – eine multizentrische Analyse

Muster der Bronchoalveolärlavage bei fibrotischen und nicht-fibrotischen interstitiellen Lungenerkrankungen – eine multizentrische Analyse

- [Evgeni German 1](#), [Ophir Freund](#), [Ori Wand](#), [Sonia Schneer](#), [Jazmin Bloch](#), [Tzvil Hershko](#), [Eyal Kleinhendler](#), [Doron Cohn-Schwartz](#), [Yitzhak Hadad](#), [Galit Aviram](#), [Yochai Adir](#), [David Shitrit](#), [Amir Bar-Shai](#) & [Avraham Unterman](#)

Wir bieten eine unbearbeitete Version dieses Manuskripts, um frühzeitigen Zugang zu seinem Ergebnisse. Vor der endgültigen Veröffentlichung wird das Manuskript weiter bearbeitet. Bitte beachten Sie Es können Fehler vorliegen, die den Inhalt beeinträchtigen, und alle rechtlichen Haftungsausschlüsse gelten.

Abstrakt

Hintergrund

Bronchoalveoläre Lavage (BAL) zelluläre Analyse wird als Teil der diagnostischen Aufarbeitung von interstitiellen Lungenerkrankungen (ILD) empfohlen, wenn die Diagnose unklar ist. In den letzten Jahren wurde ILD aufgrund des Vorhandenseins oder Fehlens von Fibrose klassifiziert. Ziel dieser Studie war es, BAL-Zellprofile über mehrere ILDs zu vergleichen, die durch den Fibrose-Status geschichtet sind.

Methoden

Eine retrospektive multizentrische Studie mit aufeinanderfolgenden Patienten, die zwischen 2017 und 2022 Bronchoskopie, einschließlich BAL für die ILD-Bewertung, unterzogen wurden. BAL-Indizes wurden auf der Grundlage von Richtlinien und früheren Forschungen ausgewählt. Brustradiologen, die für klinische Daten geblendet waren, führten eine radiologische Beurteilung durch. ILD-Diagnosen wurden durch multidisziplinäre Diskussion zugewiesen.

Ergebnisse

Insgesamt wurden 238 Patienten mit BAL-Ergebnissen eingeschlossen (Mittelalter 60 Jahre, 46% Frauen, 50,4% mit fibrotischer ILD, 91% mit gleichzeitiger transbronchialer Biopsie). BAL-Lymphozyten > 20% und > 30% waren im nicht-fibrotischen Vergleich zu fibrotischer ILD häufiger (36,4% vs. 17,5% und 25,4% vs. 9,2%, $p < 0,01$). Im multivariaten linearen Regressionsmodell war die fibrotische Erkrankung mit niedrigeren Lymphozytenzahlen assoziiert ($\beta = -6,15$, $p = 0,007$). Die Raten von gemischten BAL-Mustern, Lymphozytose oder isolierten Neutrophilen > 3% unterschieden sich nicht zwischen fibrotischen Erkrankungen. In der Computertomographie war die BAL-Lymphozytose nicht mit Bodenglastrübungen oder Mosaikdämpfung sowohl in fibrotischen als auch in nicht-fibrotischen Gruppen assoziiert, während die Wabenbildung mit einer niedrigeren Rate erhöhter (> 4,5%) BAL-Neutrophilen (71 % vs. 91 %, $p = 0,018$) verbunden war.

Schlussfolgerung

Spezifische Muster in BAL Differential waren in fibrotischer ILD selten und waren nicht unterschiedlich unter seinen Subtypen. Als eigenständige Modalität scheint BAL einen begrenzten Nutzen in der fibrotischen ILD zu haben.

Abkürzungen

BAL: Bronchoalveoläre Lavage

ILD: Interstitielle Lungenerkrankung

Erklärung zu Interessenkonflikten

A.U. berichtet, dass sie Forschungsmittel von Boehringer Ingelheim und der Pulmonary Fibrosis Foundation, persönliche Beratungsgebühren von Boehringer Ingelheim, RemedyCell, Splisense, Augmanity Nano und 1E Therapeutics in den letzten 36 Monaten erhalten haben, alle außerhalb der eingereichten Arbeit. Y.A meldet persönliche Gebühren von Teva, Zuschüsse und persönliche Gebühren von GSK und AstraZeneca sowie persönliche Gebühren von Sanofi, BI und Kamada, außerhalb der eingereichten Arbeit. A.B.S-Berichte, die persönliche Beratungsgebühren und Vorlesungsgebühren von Sanofi-Regeneron, Astrazeneca, GSK, Kamada, Boehringer Ingelheim, Roche, außerhalb der eingereichten Arbeiten erhalten. Alle anderen Autoren berichten von keinem Interessenkonflikt.