Word count: Text 3284, Abstract 140

Bronchoalveolar lavage differential cell count on prognostic assessment of patients with stable or acute interstitial lung disease: A retrospective real-life study

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ABSTRACT

BAL cell differential counts of 133 therapy naive ILD patients and 43 patients during acute exacerbation of ILD (AE-ILD) were retrospectively evaluated.

In the 20 patients who underwent BAL both at baseline and during an AE-ILD, there was an increase in neutrophils but a decrease in macrophages and eosinophils in the BAL obtained during AE-ILD. A detectable number of basophils at the baseline was a novel risk factor for earlier death and the occurrence of AE-ILD. Total BAL cell count >$160x10^9$/L during AE-ILD was correlated with a more favorable prognosis. BAL cell counts <20% of lymphocytes or >20% of neutrophils during AE-ILD were associated with shorter survival.

AE-ILD exerted significant changes in BAL cell profiles in individual patients. Several BAL-parameters correlated with survival of ILD patients; of these, baseline basophils and total cell count during AE-ILD were novel prognostic markers.

Keywords: Interstitial lung disease, idiopathic pulmonary fibrosis, acute exacerbation, bronchoalveolar lavage, prognosis.

1. INTRODUCTION

Analysis of cell differential counts in bronchoalveolar lavage (BAL) fluid has been regarded as useful, though not in itself sufficient, in the diagnosis of specific types of interstitial lung diseases (ILD) [1]. According to the current international guidelines on the diagnosis of IPF, BAL is recommended to be performed if high-resolution computed tomography (HRCT) reveals other patterns than definite usual interstitial pneumonia (UIP), namely probable UIP, indeterminate for UIP or some alternative diagnosis [2].

There is limited data on how valuable BAL is as a prognostic tool or its usefulness in predicting the response to therapy [1]. However, some previous studies have shown that BAL might be beneficial in risk prediction in terms of the occurrence of AE-ILD or mortality in different types of ILD, as presented in Table 1 [3–11], which also lists four studies which have described BAL cell differential counts examined during an AE-ILD [12–15].

(Table 1 here)

The importance of BAL in the diagnostics of AE-ILD has been a subject of debate in recent years. In addition to typical symptoms and new, bilateral alveolar changes in computed tomography (CT), the previous international recommendation on AE-IPF demanded the exclusion of infection which had to be performed by collecting either BAL or an endobronchial aspirate [16]. The present guideline does not recommend performing BAL during AE-IPF, since distinguishing an infection triggered AE-IPF from idiopathic cases is not supported by research data [17]. Even though the guidelines on AE were made originally for IPF, they have also been applied to other AE-ILDs with progressive phenotypes [18].

Abbreviations: AE, acute exacerbation; AE-ILD, acute exacerbation of ILD; AE-BAL, bronchoalveolar lavage obtained during AE-ILD; BAL, bronchoalveolar lavage; CT, computed tomography; DLCO, diffusion capacity for carbon monoxide; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; MGG, May-Grünwald-Giemsa; PAPA, Papanicolaou; UIP, usual interstitial pneumonia; TCC, total cell count.

The aim of our study was to evaluate BAL differential cell counts in ILD patients at the time of the initial ILD diagnosis or/and AE-ILD. We investigated whether different BAL cellular profiles were associated with mortality and the risk and outcome of AE-ILD.

2. MATERIALS AND METHODS

2.1. Patient and data collection

The study cohort consists of IPF and other ILD patients hospitalized in Oulu University Hospital and Oulaskangas Hospital in Northern Finland between 1/1/2008 and 31/12/2017. A total of 89 patients also belonged to the cohort of our recently published study [19]. The patients' data were collected from the hospital medical records by performing a search with the International Classification of Diseases version 10 codes J84.1, J84.8, J84.9. J61, J99, J99.0* and J99*M05.1. The patients with non-elective hospitalizations due to acute respiratory symptoms were collected and separated into two groups, namely those who had an AE-ILD and those not experiencing this event.

The type of ILD was re-evaluated using the current international criteria as described in our previous study [2, 19-20]. ILD patients with a non-fibrotic pattern in high-resolution computed tomography of chest were excluded, e.g. patients with organizing pneumonia or non-fibrosing parenchymal sarcoidosis. The histological diagnoses from surgical lung biopsies and autopsies were reviewed. Patients with the pattern of probable or consistent with usual interstitial pneumonia (UIP) in high-resolution computed tomography (HRCT) according to the present classification or a histopathological finding of UIP were classified as UIP patterns. The most recent definition of AE-IPF was applied to all other ILDs as well [17].

The final study material consists of patients who had undergone bronchoscopy with BAL either at initial diagnosis (baseline BAL) and/or during AE-ILD (AE-BAL), as presented in Figure 1. The baseline BAL, which was performed in stable patients in the outpatient clinic, was included in the analysis if the patient had not received pharmacotherapy for ILD before the procedure and had neither a lower respiratory tract infection nor an AE-ILD at the time of the procedure. Fourteen patients were diagnosed with ILD during AE, when they also had undergone their first bronchoscopy. The BAL cell differential counts of these 14 patients were classified as AE-BALs. Information on the pharmacotherapy for ILD preceding the hospitalization and that used for AE-ILD was collected from all the files of those patients who had provided an AE-BAL.

The clinical information was collected systematically from electronic patient records available for more than 20 years in the study hospitals. Pulmonary function test results were recorded, if they were available within a 6 months' time range before or after the BAL procedure date. Age and follow-up time were calculated using the BAL procedure date and death, transplantation or last follow-up date (31/8/2019). Death dates were collected from death certificates housed in the national registry of Statistics Finland. Patients with less than 5 pack-years of smoking history were regarded as non-smokers.

2.2. BAL procedure

Both BAL procedures in the bronchoscopy unit as well as the preparation of BAL samples in the pathology department have been performed in a systematic manner with accurate recording for several decades. The BAL procedure has been described in detail previously [21]. The selected segment for baseline BAL was in the right middle lobe (n=99), left lower lobe (n=13), right lower lobe (n=10), right

upper lobe (n=6), left upper lobe (n=1) or lingula (n=1). In three patients, the data describing the baseline BAL region was not available. The site of AE-BAL had been recorded in 36 of 43 patients; of these, the selected segment was in the right middle lobe (n=20), right lower lobe (n=9), left lower lobe (n=5), right upper lobe (n=1) or lingula (n=1). A syringe had been attached directly into the working channel of the bronchoscope. Aliquots of 20 mL of sterile saline at 37°C had been instilled into a lung segment and the fluid was aspirated manually with the syringe after each instillation. A total volume of 200 ml had been used for the lavage in most cases with the average recovered volumes being 123 mL in baseline BAL samples and 121 mL in AE-BAL samples. The BAL fluid collected was stained with Papanicolaou (PAPA) and May-Grünwald-Giemsa (MGG) for total and differential cell counts. In addition to percentage of cells from the total cell count (TCC), the total numbers of inflammatory cells were calculated as suggested by an earlier study [22]. The pathologists' reports of BAL fluid were available from all included study subjects. Bronchial and/or blood contamination was reported in four baseline BAL samples and 8 AE-BAL samples. However, also these samples were analyzed and differential cell count results were reported as routinely.

2.3. Ethical issues

As this was a register-based retrospective study and the majority of study subjects were deceased, no patient consent forms were gathered in accordance with Finnish legislation. The study protocol was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District (statement 2/2015). Permission to use death certificates was given by Statistics Finland (Dnro: TK-53-515-15). The study was conducted in compliance with the Declaration of Helsinki.

2.4. Statistical analysis

IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp.) was used to perform statistical analysis and Origin(Pro), Version 2019b (OriginLab Corporation, Northampton, MA, USA) was utilized for graphs. Means and standard deviation were calculated for continuous variables, which were normally distributed. Medians and range (interquartile or minimum–maximum) were utilized for continuous variables which were not normally distributed. Group differences of continuous variables were tested by using independent samples *t*-test and one-way ANOVA followed by Tukey's test, paired samples *t*-test, Mann-Whitney U-test or Wilcoxon Signed Ranks test, when appropriate. Group differences for categorical variables were evaluated by using Chi-Square or Fisher's Exact test. Survival and AE-ILD-free time from diagnosis date were estimated by using Kaplan-Meier curves and the groups were compared with each other by using log rank tests. Cox regression was used for univariate and multivariate analyses, in which adjustments for gender, age, smoking, pulmonary function test results and ILD type (IPF vs. non-IPF) were made. The cut point values for different BAL cell subtypes were determined by utilizing medians, quartiles or quintiles of each cell subtype depending on the range of each cell count.

3. RESULTS

3.1. Study subjects

We examined 133 therapy naive ILD patients for whom we had baseline BAL data and in addition, there were 43 patients in whom BAL had been obtained during AE-ILD (Table 2); of these, 20 patients had undergone two consecutive BALs, e.g. the first specimen had been gathered at time of the initial diagnosis and the second later during an AE-ILD. One out of 20 patients had baseline BAL differential

cells analyzed only with PAPA staining. The majority of the subjects suffered from IPF, while the most common non-IPF ILDs were connective tissue disease-associated ILD, non-specific interstitial pneumonia and asbestosis. When the cases were categorized according to the UIP pattern, all IPF patients revealed UIP, while 52 % of non-IPF patients with baseline BAL and 26 % of non-IPF patients with AE-BAL had the UIP pattern. The characteristics of the study subjects are presented in Table 3 and pharmacological therapy of the patients with AE-BAL data is presented in Table A.1.

Almost every second subject i.e. 66 out of 133, patients with baseline BAL experienced an AE-ILD during the follow-up time. None of these patients had AE triggered by bronchoscopy or the collection of BAL. In those having undergone BAL during the AE-ILD, most patients had no identifiable trigger for the AE-ILD; a respiratory infection was the trigger in 10 patients out of 43 (Table A.2).

Table 2. Study patients with interstitial lung disease.

Type of ILD		Baseline BAL	BAL obtained during AE-ILD		
			Total	Both baseline BAL and AE-BAL	Only AE-BAL
		N=133	N=43	N=20	N=23
IPF		81 (61)	24 (56)	14 (70)	10 (44)
NSIP		12 (9.0)	8 (19)	2 (10)	6 (26)
Asbestosis		15 (11)	3 (7.0)	2 (10)	1 (4.3)
CHP		5 (3.8)	0	0	0
CTD-ILD		16 (12.0)	7 (16)	2 (10)	5 (22)
	RA	13 (9.8)	6 (14)	2 (10)	4 (17)
	PM	1 (0.8)	0	0	0
	SLE	1 (0.8)	0	0	0
	SSj	1 (0.8)	0	0	0
	SSc	0	1 (2.3)	0	1 (4.3)
Unclassifiable ILD		3 (2.3)	1 (2.3)	0	1 (4.3)
DIP		1 (0.8)	0	0	0

Data is presented as the number of patients (%). AE-BAL, bronchoalveolar lavation obtained during acute exacerbation of ILD; AE-ILD, acute exacerbation of interstitial lung disease; BAL, bronchoalveolar lavage; CHP, chronic hypersensitivity pneumonitis; CTD-ILD, connective tissue disease-associated

interstitial lung disease; DIP, desquamative interstitial pneumonia; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; PM, polymyositis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SSj, Sjögren's syndrome.

Table 3. Demographics of the study subjects.

Characteristic	Baseline BAL	BAL obtained during AE-ILD			
		Total	AE-BAL without baseline BAL	Baseline BAL and AE-BAL	p-value[A]
	N=133	N=43	N=23	N=20	
Age, years	68±9.9	68±11	70±11	66 ±11	0.176
Male	80 (62)	30 (70)	15 (65)	15 (75)	0.486
Smoking status at BAL procedure					
Non-smoker	67 (50)	20 (47) [E]	14 (64)	6 (30)	0.029
Ex-smoker	53 (40)	20 (47)	6 (27)	14 (70)	0.006
Current smoker	13 (9.8)	2 (4.7)	2 (9.1)	0	0.489
Pack-years of ever-smokers[B]	26±16	25±14	21±13	27±14	0.374
FVC%	75±15[C]	64±17 [F]	69±15	58±18	0.063
FEV1%	80±16[C]	67±17 [F]	72±15	62±18	0.089
DLCO%	55±17[D]	41±14 [F]	41±15	41±14	0.962
AE-ILD during follow-up	66 (50)	43 (100)	23 (100)	20 (100)	>0.999
Deceased or transplanted	116 (87)	36 (84)	18 (78)	18 (90)	0.420
Transplanted during follow-up	6 (4.5)	2 (4.7)	0	2 (10)	0.210
Usual interstitial pneumonia	108 (81)	29 (67)	12 (52)	17 (85)	0.022

Data is presented as mean± standard deviation and number of patients (%) when appropriate. P-values were calculated by using *t*-test or Chi-Square test. Age, smoking and pulmonary function data are related to the BAL procedure date. AE-BAL, bronchoalveolar lavage obtained during an acute exacerbation of ILD; AE-ILD, acute exacerbation of interstitial lung disease; BAL, bronchoalveolar lavation; DLCO, diffusion capacity for carbon monoxide; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity. [A] P-value between the patients with AE-BAL without baseline BAL and patients with both BAL samples,[B] Pack-year data of 5 patients was missing, [C] Data of 10 patients was not available, [D] Data of 12 patients was not available, [E] Smoking data of one patient was missing, [F] Data of 5 patients was not available.

3.2. BAL cell profile differences

The proportions of BAL cells at baseline and during AE-ILD are presented in Tables A.3 and A.4. IPF patients had a more neutrophilic and a more eosinophilic, but a less lymphocytic BAL cell pattern at baseline in comparison with non-IPF patients.

In the patients who had undergone bronchoscopy and BAL both at baseline and during AE-ILD, it was possible to discern significant changes in the BAL cell profiles between these two examinations (Table 4); i.e. macrophage and eosinophil counts decreased, while lymphocyte and neutrophil counts increased.

Table 4. BAL cell counts in patients with data available both at baseline and at acute exacerbation.

BAL (MGG)	BAL at baseline N=19	BAL at acute exacerbation N=19	P-value
Total cell count (10^6/l)	220.2±112.3	178.0±186.5	0.443
Macrophages (%)	72.8±15.6	38.9±22.3	<0.001
Macrophages (10^6/l)	166.5±109.5	53.7±44.9	0.002
Lymphocytes (%)	15.5±13.6	21.4±18.2	0.194
Lymphocytes (10^6/l)	30.5±29.8	36.5±45.7	0.618
Neutrophils (%)	6.76±5.09	37.3±28.6	<0.001
Neutrophils (10^6/l)	13.0±10.1	83.5±149.8	0.048
Eosinophils (%)	3.00 (1–6.50)	1.00 (0.00 –2.00)	0.041
Eosinophils (10^6/l)	8.64 (1.70–12.3)	1.10 (0.00–5.04)	0.035

Data is presented as means ± standard deviation or medians (interquartile range). P-values were calculated by using paired samples *t*-test or Wilcoxon Signed Ranks test, when appropriate. BAL, bronchoalveolar lavage; MGG, May-Grünwald-Giemsa.

3.3. BAL cell profiles and survival

Median survival of the study subjects with baseline-BAL data available was 5.5 years (95% CI 4.5-6.6). Basophils were observed in baseline BAL samples from 17 IPF patients and 10 non-IPF patients.

Basophils were associated with mortality and a shorter AE-ILD-free time in all study subjects (Figure 2, Table 5), and the result was not affected by confounding factors in a multivariate analysis. These associations were seen only in non-IPF patients in the further analysis. In non-IPF patients, basophils were revealed to be a non-independent risk factor for shorter survival and an independent risk factor for the occurrence of an AE-ILD in multivariate analysis.

Median survival of the patients with AE-ILD after the BAL procedure date was 18 months (95% CI 0.0-38.1). Thirty-day mortality after AE-BAL was 26 % and 90-day mortality was 42 % (Table A.2). The univariate analysis of the AE-BAL findings and risk for mortality is presented in Table 5. Patients with TCC more than $160x10^6$/l had a longer median survival of 57.9 months (95%CI 26.1-89.6) compared with patients with TCC $160x10^6$/l or less, whose median survival was 1.5 months (95%CI 0.0-4.1) (Figure 3(A)). Patients who had a 20 % or higher lymphocyte count in AE-BAL had a longer median survival of 58 months as compared with 2.9 months in patients with lymphocytes fewer than 20 % (Figure 3(C)). In contrast, higher proportion of neutrophils in AE-BAL was associated with a shorter survival i.e. patients with a more than 20 % neutrophil count had a median survival of 1.0 months (95%CI 0-5.8) as compared to 41 months (95%CI 16.9-64.8) in patients with less neutrophils (Figure 3 (C)). The abovementioned results were not significantly changed in the multivariate analysis by confounding for gender, age, smoking, forced vital capacity or diffusion capacity for carbon monoxide. However, after adjustment for the ILD types (IPF vs. non-IPF), low TCC was found to be the only independent risk factor for shorter survival. In addition, the number of macrophages was associated with a more favorable prognosis, although this could not be detected with the percentages of macrophages. In further analyses, TCC of AE-BAL was not associated with survival when tested separately for IPF and non-IPF. In contrast, an AE-BAL lymphocyte count of less than 20 % and AE-BAL neutrophils higher than 20 %

were found to be independent risk factors for death in non-IPF patients, but not in IPF subjects. Furthermore, the number of AE-BAL macrophages correlated with a more favorable prognosis only in non-IPF patients. There were no correlations between the numbers of eosinophils or basophils in AE-BAL with survival.

Table 5. Univariate analysis of baseline BAL and AE-BAL findings.

	All		IPF		Other ILD	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Risk for mortality						
BAL cell count						
BAL Baso > 0%	1.58 (1.01–2.46)	0.045	1.18 (0.68–2.03)	0.555	2.70 (1.22–5.95)	0.014
BAL Baso No[A]	1.16 (1.02–1.33)	0.026	1.09 (0.94–1.27)	0.254	1.71 (1.11–2.63)	0.015
Risk for AE-ILD						
BAL Baso > 0%	1.79 (1.02–3.13)	0.043	1.27 (0.62–2.60)	0.506	3.49 (1.36–8.93)	0.009
BAL Baso No[A]	1.21 (1.06–1.38)	0.004	1.16 (0.99–1.37)	0.070	2.12 (1.28–3.50)	0.003
Risk for mortality during AE-ILD						
AE-BAL total cell count <160E6/l	3.01 (1.47–6.16)	0.003	2.01 (0.80–5.04)	0.135	2.54 (0.75–8.58)	0.133
AE-BAL Lymph < 20 %	2.49 (1.20–5.16)	0.015	1.01 (0.39–2.60)	0.980	4.11 (1.09–15.46)	0.036
AE-BAL Lymph No [A]	0.99 (0.98–1.00)	0.046	1.00 (0.98–1.02)	0.678	1.00 (0.98–1.01)	0.393

AE-BAL Neut > 20 %	2.53 (1.29–4.95)	0.007	1.12 (0.48–2.64)	0.788	5.17 (1.45–18.35)	0.011
AE-BAL Neut No [A]	1.00 (1.00–1.00)	0.478	1.00 (1.00–1.00)	0.509	1.02 (1.01–1.03)	0.008
AE-BAL Mac No [A]	0.99 (0.98 –1.00)	0.005	1.00 (0.99–1.00)	0.277	0.98 (0.97–1.00)	0.017

[A]Absolute number of cells (10^6/l)

AE-BAL, bronchoalveolar lavage obtained during acute exacerbation of interstitial lung disease; BAL, bronchoalveolar lavage; Baso, basophils; CI, confidence interval; Eos, eosinophils; HR, hazard ratio; IPF, idiopathic pulmonary fibrosis; Lymph, lymphocytes; Mac, macrophages; MGG, May-Grünwald-Giemsa; Neut, neutrophils.

4. DISCUSSION

We have examined a relatively large real-life material of BAL cell differential counts of IPF and non-IPF patients, with and without an AE-ILD. We found significant changes in BAL cell profiles caused by AE-ILD. We were also able to detect a correlation of AE-BAL TCC with survival and several correlations between BAL cell differential counts and survival time.

BAL cell profiles showed a more neutrophilic and lymphocytic pattern in AE-ILD than in the stable phase of the disease, while macrophages and eosinophils displayed an opposite tendency. Similar observations have been presented in two previous publications [12–13]. We were able to detect significant changes in BAL cell differential counts between the baseline BAL samples and AE-BALs in 19 patients in whom we had both samples available, a finding which has not been described earlier. Previous studies have compared BAL cell profiles between the study subjects with either baseline-BAL or AE-BAL, but not detected changes in BAL cell profiles in individual patients having undergone both of these BAL examinations [12–13]. Our results could be verified both by using percentages of each cell

type and by using total numbers of differential cells. One may speculate that these results reflected the significant changes occurring in the alveolar inflammatory cell counts during an AE-ILD.

We are the first investigators to demonstrate that basophils are a prognostic marker for survival and a risk factor for AE-ILD by using the total number of basophils in addition to percentages. There is one previous study describing an association of BAL procollagen-III-levels and BAL basophils in IPF patients, but this was not correlated with survival [23]. Furthermore, both *in vitro* and in mouse experiments, it has been recently demonstrated that basophils have a significant role in the development and function of alveolar macrophages in controlling lung homeostasis [24]. Our results suggest that this cell type and its role as a prognostic marker should be clarified in the future.

A clear correlation with AE-BAL cell profiles and survival after AE-ILD was detected here, with TCC more than 160 x 10^6/l, lymphocytes and macrophages being associated with longer and neutrophils with shortened survival. In a previous study, significant differences in BAL TCC between ILD types could be observed [22]. However, our study is the first to suggest some prognostic significance of the TCC in the BAL. The association of a high lymphocyte count with a more favorable prognosis in AE-ILD patients has been detected in two earlier studies, of which one included only IPF patients and the other also non-IPF ILD patients, thus confirming our results [4,7]. Even though we were not able detect a statistically significant difference in survival time and lymphocyte count in IPF patients when non-IPF patients were excluded from the analysis, the association of lymphocytes with survival was clear when all AE-ILD patients were included into analysis. This might suggest that the effect of lymphocytes on survival is similar, although less clear, in IPF as it is in non-IPF patients, but the number of study subjects with AE-IPF evaluated here was too small to reach statistical significance. The association of high neutrophil count with shortened survival has been found in two studies investigating stable IPF patients [3,8]. In

contrast, Tabuena et al. stated that the absolute number of neutrophils was associated with a decreased relative risk for death in IPF patients [10]. As far as we are aware, our study is the first to show that AE-BAL neutrophil counts are an independent risk factor for shortened survival in non-IPF patients. The total number of macrophages in AE-BAL has not been examined earlier as a potential prognostic factor, even though a recent study noted that percentages of macrophages correlated with shorter survival in stable IPF patients [8].

In the current guidelines, bronchoscopy and BAL are not recommended for differential diagnostic purposes between lower respiratory tract infections and AE-IPF [17]. The safety of the procedure during AE-ILD has also been an issue of concern. It was reported that performing bronchoscopy and BAL during AE-ILD did not provide any useful information for treatment decisions in a cohort of 119 patients [14]. However, that study did not include a control group of AE-ILD patients not having undergone bronchoscopy and thus, it did not provide data on the possible effects of the procedure itself on outcome. In the study of Takei et al. (2017) none of the 37 AE-ILD patients who had undergone bronchoscopy suffered from severe worsening of AE after the bronchoscopy [7]. In our study, 30-day mortality after AE-BAL date was 26 % and 90-day mortality was 42 %, these results being in line with other reports describing AE-ILD patients not undergoing routine bronchoscopy [25–27]. In addition, we could not detect any AE-ILDs triggered by a bronchoscopy performed for diagnostic purposes at baseline, a result supported by earlier studies in which AE-IPF occurred instantly after BAL only in 3 of 124 [28] and 1 of 147 IPF patients [29]. Our results, together with the observations mentioned above, do suggest that BAL is a relatively safe procedure also during an AE-ILD.

An interesting finding emerging from our study was the higher number of columnar epithelial cells in AE-BAL samples as compared to those collected in the stable phase of the disease. The average volume

of recovered BAL fluid obtained during AE-ILD did not differ from the baseline BAL samples, indicating that both sample types represented equally well the alveolar space. One may speculate that high columnar epithelial cell counts might not be a sign of poor standards of the BAL fluid sample, but rather a consequence of the lung injury, which has possibly affected bronchiolar airway epithelium in addition to the alveolar space. Our results are implicitly supported by those of a recent study, which analyzed the transcriptome of BAL samples from IPF patients and showed that genes associated with mortality in BAL cells were enriched in those genes expressed in airway basal cells [30]. Clearly, the role of airway epithelial cells in BAL deserves further investigation.

A retrospective study design and the heterogeneity of the non-IPF study subjects can be regarded as limitations of our study. Nevertheless, it should be considered that implementing a prospective real-life study in this setting would be challenging, because the present guidelines allow for the diagnosis of IPF without performing bronchoscopy and BAL if the radiological pattern is typical for UIP [2]. Our study included only those ILD patients, who were non-electively hospitalized at some phase of their disease history, which may have caused some bias. However, the baseline BAL data was collected from the patients of the outpatient clinic in the stable phase of the disease before the non-elective hospitalization. It should also be noticed that hospitalization of an ILD patient is quite a probable event, at least in the terminal phase of the disease, i.e. the fact is that most ILD patients will die because of their ILD [31–34]. We think we were able to collect a rather comprehensive material, which is a consequence of the detailed manner of BAL reporting and the systematic way of performing BAL procedures and preparation of samples in our hospital.

5. CONCLUSIONS

To conclude, we detected significant changes in BAL-cell profiles in patients having undergone both BAL at baseline and AE-BAL. We detected several new prognostic markers, namely basophils in the stable phase of the ILD, and TCC, number of macrophages and BAL neutrophil count during AE-ILD. As a result, BAL might be a more functional tool in the prognostic evaluation of ILD patients than previously believed.

Acknowledgements

The authors would like to thank for Dr. Ewen MacDonald for language and editorial assistance.

Declaration of interest: JS reports congress fees and travel subsidy from Boehringer-Ingelheim, GlaxoSmithKline, Novartis, Orion Pharma, Ratiopharm and Roche, and lecture fees from Boehringer-Ingelheim, Chiesi, GlaxoSmithKline, Mundipharma, Orion Pharma and Roche outside the submitted work. EK reports congress fee and travel subsidy from Orion Pharma outside the submitted work. HL and HV have nothing to disclose. MP reports a lecture fee from Boehringer-Ingelheim Finland Ltd and a congress fee and travel subsidy from Roche, outside the submitted work. RK reports consultant fees from Boehringer-Ingelheim, lecture fees from Roche and Boehringer-Ingelheim, and a congress travel subsidy from Orion pharma outside the submitted work.

Funding source declaration: This work was supported by the Foundation of the Finnish Anti-Tuberculosis Association, Ryttylä, Finland; Research Foundation of the Pulmonary Diseases HES, Helsinki, Finland; Jalmari and Rauha Ahokas Foundation, Helsinki, Finland and the Research Foundation of North Finland, Oulu, Finland. The funding sources were not involved in the study design, collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the article for publication.

Submission declaration and verification: This work has not been published previously, is not under consideration for publication elsewhere, is approved by all authors and by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Authors' contributions: JS collected the study material and interpreted and analyzed the data. JS and HL prepared the draft of the manuscript. HL participated substantially in the BAL data collection. EK participated in planning BAL data collection form, provided methodological assistance concerning the BAL procedure and participated in the interpretation of the data. HV planned and participated in the statistical analysis. RK and MP participated in planning the data collection, study design and in the interpretation of the data. RK managed the study and contributed substantially to data interpretation by re-evaluating the study patients and data from medical records. All authors participated in the preparation of the manuscript, read and approved the final manuscript.

Data statement: The datasets generated and analyzed during the current study are not publicly available due to the relatively small population of Northern Finland since we could not guarantee individuals' anonymity as the data was collected in a detailed manner, but it is available from the corresponding author on reasonable request.

RFEFERENCES

1. 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;185:1004–14

2. 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;198:e44–e68.

3. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest. 2008;133:226–32.

4. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J. 2011;37:356–63.

5. Arai T, Kagawa T, Sasaki Y, Sugawara R, Sugimoto C, Tachibana K, et al. Heterogeneity of incidence and outcome of acute exacerbation in idiopathic interstitial pneumonia. Respirology. 2016;21:1431–7.

6. Kakugawa T, Sakamoto N, Sato S, Yura H, Harada T, Nakashima S, et al. Risk factors for an acute exacerbation of idiopathic pulmonary fibrosis. Respir Res. 2016;17:79.

7. Takei R, Arita M, Kumagai S, Ito Y, Noyama M, Tokioka F, et al. Impact of lymphocyte differential count > 15% in BALF on the mortality of patients with acute exacerbation of chronic fibrosing idiopathic interstitial pneumonia. BMC Pulm Med. 2017;17:67.

8. Song H, Sun D, Ban C, Liu Y, Zhu M, Ye Q, et al. Prognosis of patients with idiopathic pulmonary fibrosis. Med Sci Monit. 2019:25:4194–01

9. Boomars KA, Wagenaar SS, Mulder PG, Velzen-Blad H, van den Bosch JM. Relationship between cells obtained by bronchoalveolar lavage and survival in idiopathic pulmonary fibrosis patients. Throrax 1995;50:1087–92.

10. Tabuena RP, Nagai S, Tsutsumi T, Handa T, Minoru T, Mikuniya T, et al. Cell profiles of bronchoalveolar lavage fluid as prognosticators of idiopathic pulmonary fibrosis/usual interstitial pneumonia among Japanese patients. Respiration. 2005;72:490–8.

11. Ruy YJ, Chung MP, Han J, Kim TS, Lee KS, Chun E-M, et al. Bronchoalveolar lavage in fibrotic idiopathic interstitial pneumonias. Respir Med. 2007;101:655–60.

12. Lee JS, Song JW, Wolters PJ, Elicker BM, King Jr TE, Kim DS, et al. Bronchoalveolar lavage pepsin in acute exacerbation of idiopathic pulmonary fibrosis. Eur Respir J. 2012;39:352–8.

13. Schupp JC, Binder H, Jäger B, Cillis G, Zisse G, Müller-Quernheim J, et al. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. PLoS ONE. 2015;10:e0116775.

14. Arcadu A, Moua T. Bronchoscopy assessment of acute respiratory failure in interstitial lung disease. Respirology. 2017;22:352–9.

15. Yamazaki R, Nishiyama O, Saeki S, Sano H, Iwanaga T, Tohda Y. The utility of the Japanese Association for Acute Medicine DIC scoring system for predicting survival in acute exacerbation of fibrosing idiopathic interstitial pneumonia. PLoS One. 2019;14:e0212810.

16. Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, King Jr TE, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007;176:636–43.

17. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis. An International Working Group Report. Am J Respir Crit Care Med. 2016;194:265–75.

18. Kolb M, Bondue B, Pesci A, Miyazaki Y, Song JW, Bhatt NY, et al. Acute exacerbations of progressive-fibrosing interstitial lung diseases. Eur Respir Rev. 2018;27:180071.

19. Salonen J, Purokivi M, Bloigu R, Kaarteenaho R. Prognosis and causes of death of patients with acute exacerbation of fibrosing interstitial lung diseases. BMJ Open Respir Res.2020;7:e000563.

20. Travis WD, Costabel U, Hansell DM, King Jr TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med. 2013;188:733–48.

21. Keskitalo E, Varis L, Bloigu R, Kaarteenaho R. Bronchoalveolar cell differential count and the number of asbestos bodies correlate with survival in patients with asbestosis. Occup Environ Med. 2019;76:765–71.

22. Domagala-Kulawik J, Skirecki T, Maskey-Warzenchowska M, Gruberk-Jaworska H, Chazan R. Bronchoalveolar lavage total cell count in interstitial lung disease – does it matter? Inflammation. 2012;35:803–9.

23. Hiwatari N, Shimura S, Yamauchi K, Nara M, Wataru H, Shirato K. Signifigance of elevated procollagen-III-peptide and transforming growth factor-β levels of bronchoalveolar lavage fluids from idiopathic pulmonary fibrosis patients. Tohoku J Exp Med. 1996;181:285–95.

389 24. Cohen M, Giladi A, Gorki A-D, Solodkin DG, Zada M, Hladik A, et al. Lung single-cell signaling
390 interaction map reveals basophil role in macrophage imprinting. Cell. 2018;175:1031–44.

391 25. Murohashi K, Hara Y, Saigusa Y, Kobayashi N, Sato T, Yamamoto M, et al. Clinical significance of
392 Charlson comorbidity index as a prognostic parameter for patients with acute or subacute idiopathic
393 interstitial pneumonias and acute exacerbation of collagen vascular diseases-related interstitial
394 pneumonia. J Thorac Dis. 2019;11:2448–57.

395 26. Enomoto N, Oyama Y, Enomoto Y, Yasui H, Karayama M, Kono M, et al. Differences in clinical
396 features of acute exacerbation between connective tissue disease-associated interstitial pneumonia
397 and idiopathic pulmonary fibrosis. Chronic Respir Dis. 2019;16:1479972318809476.
398 https://doi.org/10.1177/1479972318809476.

399 27. Suzuki A, Kondoh Y, Brown KK, Johkoh T, Kataoka K, Fukuoka J, et al. Acute exacerbations of fibrotic
400 interstitial lung diseases. Respirology. 2020;25:525–34.

401 28. Hiwatari N, Shimura S, Takishima T, Shirato K. Bronchoalveolar lavage as a possible cause of acute
402 exacerbation in idiopathic pulmonary fibrosis patients. Tohoku J Exp Med. 1994;174:379–86.

403 29. Kim DS, Park JH, Park BK, Lee JS, Nicholson AG, Colby T. Acute exacerbation of idiopathic
404 pulmonary fibrosis: frequency and clinical features. Eur Respir J. 2006;27:143–50.

405 30. Prasse A, Binder H, Schupp JC, Kayser G, Bargagli E, Jaeger B, et al. BAL cell gene expression is
406 indicative of outcome and airway basal cell involvement in idiopathic pulmonary fibrosis. Am J Respir
407 Crit Care Med. 2019;199:622–30.

408 31. Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis
409 increased in the United States from 1992 to 2003. Am J Respir Crit Care Med 2007;176:277–84.

410 32. Nurmi HM, Purokivi MK, Kärkkäinen MS, Kettunen H-P, Selander TA, Kaarteenaho RL. Variable
411 course of disease of rheumatoid arthritis-associated usual interstitial pneumonia compared to other
412 subtypes. BMC Pulm Med 2016;16:107.

413 33. Kärkkäinen M, Nurmi H, Kettunen H-P, Selander T, Purokivi M, Kaarteenaho R. Underlying and
414 immediate causes of death in patients with idiopathic pulmonary fibrosis. BMC Pulm Med 2018;18:69.

415 34. Kolb M, Vašáková M. The natural history of progressive fibrosing interstitial lung diseases. Respir
416 Res 2019;20:57.

417

418 Table 1. Previous studies examining differential cell counts in bronchoalveolar lavage (BAL) fluid.

Publication	Participants (n)	BAL cell type and correlation with risk for AE-ILD	BAL cell type and correlation with risk of mortality	BAL description during AE-ILD
Kinder et al 2008, USA [3]	156 stable IPF	NA	Neutrophil percentage (increased)	NA
Song et al 2011, Japan [4]	461 IPF, of which 96 with AE-IPF	NA	Lymphocyte percentage at AE (decreased)	NA
Arai et al 2016, Japan [5]	BAL database: 92 IPF 74 possible UIP 63 inconsistent with UIP	Neutrophil percentage (increased)	NA	NA
Kakugawa et al 2016, Japan [6]	41 stable IPF 24 AE-IPF	Eosinophils ≥3.21% (increased) Neutrophils ≥1.77% (increased)	Eosinophils ≥3.21 % (increased)	NA
Takei et al 2017, Japan [7]	37 AE-CFIIP	NA	Lymphocytes >15% (decreased)	NA
Song et al, China 2019 [8]	178 stable IPF	NA	Macrophage and neutrophil percentages (increased) Lymphocyte percentages (decreased)	NA
Boomars et al, Netherlands 1995 [9]	49 stable IPF	NA	Eosinophils percentage and number (increased)	NA
Tabuena et al, Japan 2005 [10]	81 stable IPF	NA	Number of neutrophils (decreased) Number and percentage of eosinophils (decreased)	NA

Ruy et al, South Korea, 2006 [11]	87 stable UIP 35 stable NSIP	NA	Lymphocyte percentage (decreased, only in UIP)	NA
Lee et al 2012, South Korea [12]	30 stable IPF 24 AE-IPF	NA	NA	Red blood cells and neutrophils higher during AE. Macrophages lower during AE.
Schupp et al 2015, Germany [13]	59 stable IPF 12 AE-IPF	NA	NA	Neutrophils higher during AE, lymphocytes and macrophages lower in AE.
Arcadu et al 2017, USA [14]	38 AE-IPF 37 AE of CTD-ILD 9 AE-NSIP 22 other AE-ILD	NA	NA	BAL cell counts available without comparison to stable ILD.
Yamazaki et al 2019, Japan [15]	25 AE-IPF 38 AE-CFIIP	NA	NA	BAL cell counts available without comparison to stable ILD

419 AE, acute exacerbation; CFIIP, chronic fibrosing idiopathic interstitial pneumonia; ILD interstitial lung
420 disease; IPF, idiopathic pulmonary fibrosis NA, not applicable; UIP, usual interstitial pneumonia.

421

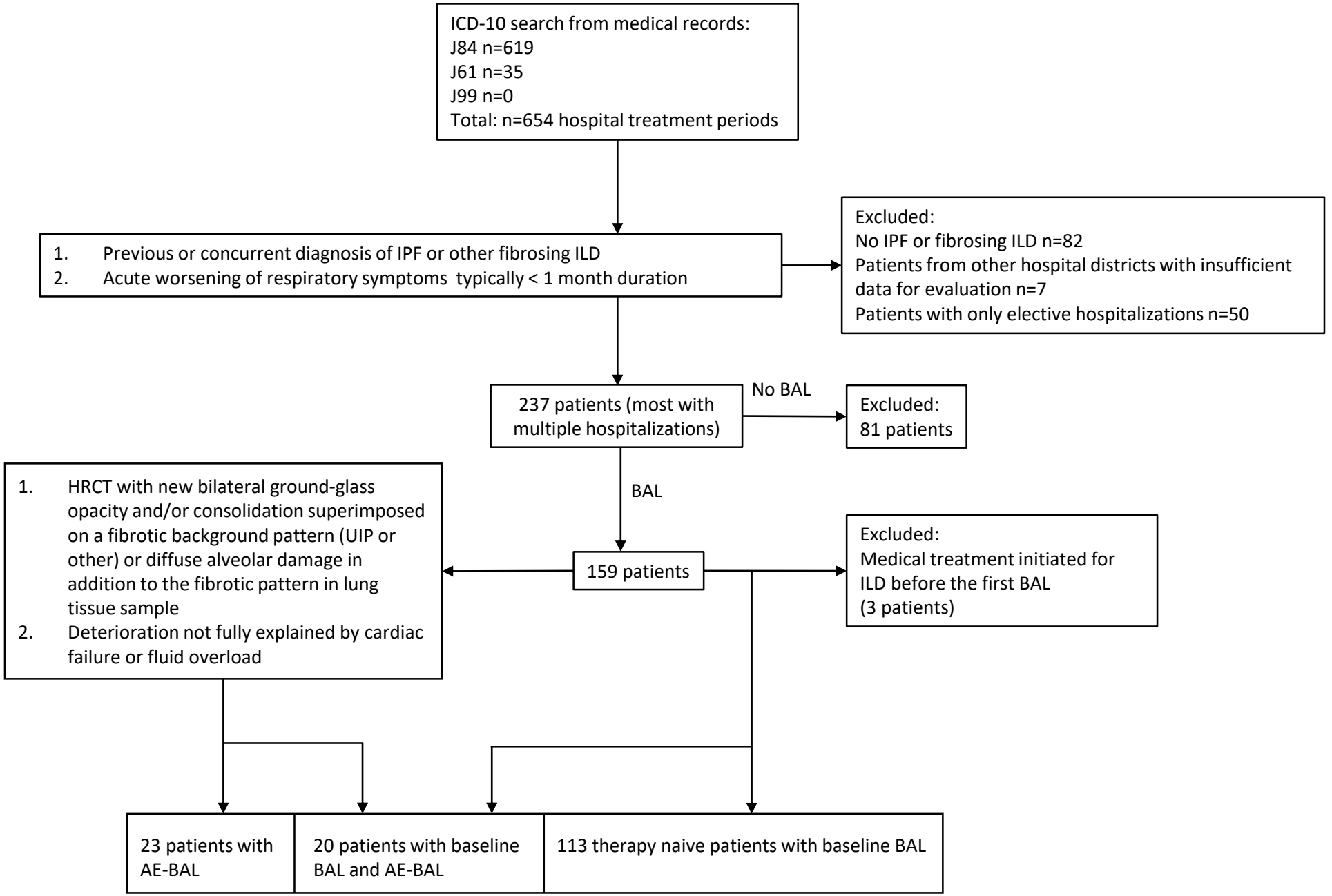
422

FIGURE LEGENDS

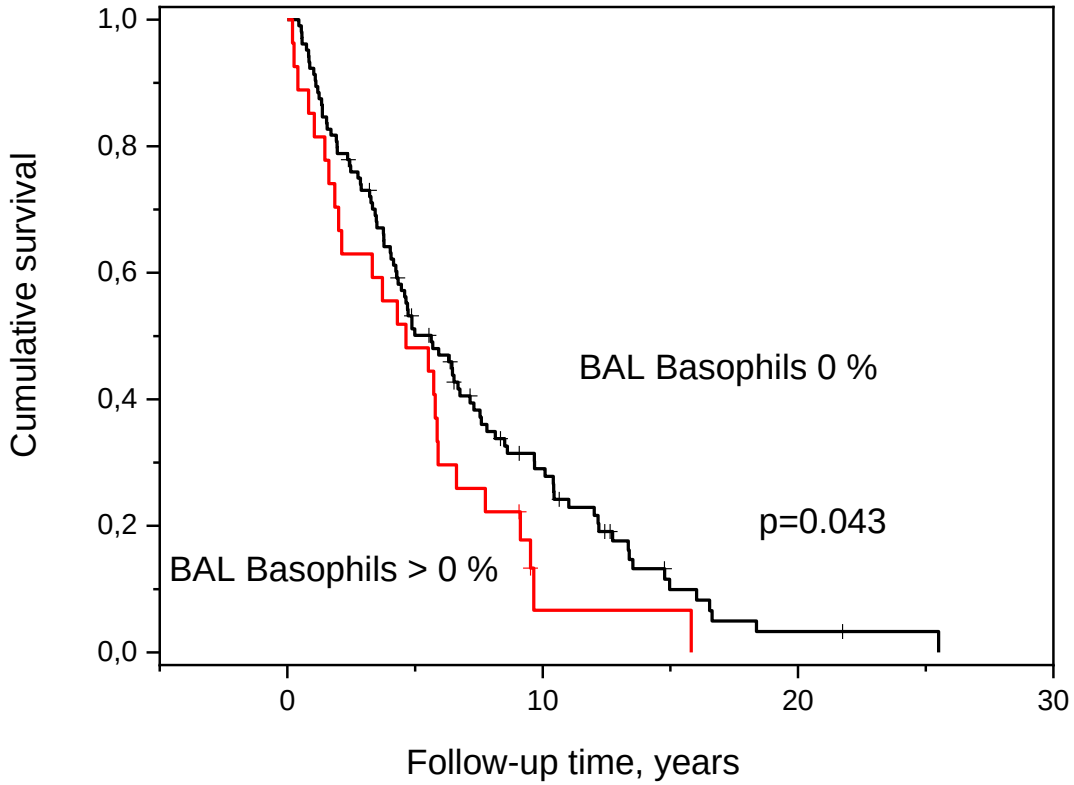
Figure 1. Flowchart of the study. AE-BAL, BAL obtained during acute exacerbation of ILD; BAL, bronchoalveolar lavage; HRCT, high-resolution computed tomography; ICD-10, International Classification of Diseases version 10; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis.

Figure 2. A) The presence of basophils in bronchoalveolar lavage (BAL) at baseline was associated with increased mortality of interstitial lung disease (ILD) (basophils > 0% n= 27, 0 % n=104). B) The presence of basophils in BAL was associated with the occurrence of AE-ILD. AE-ILD, acute exacerbation of interstitial lung disease; BAL, bronchoalveolar lavage.

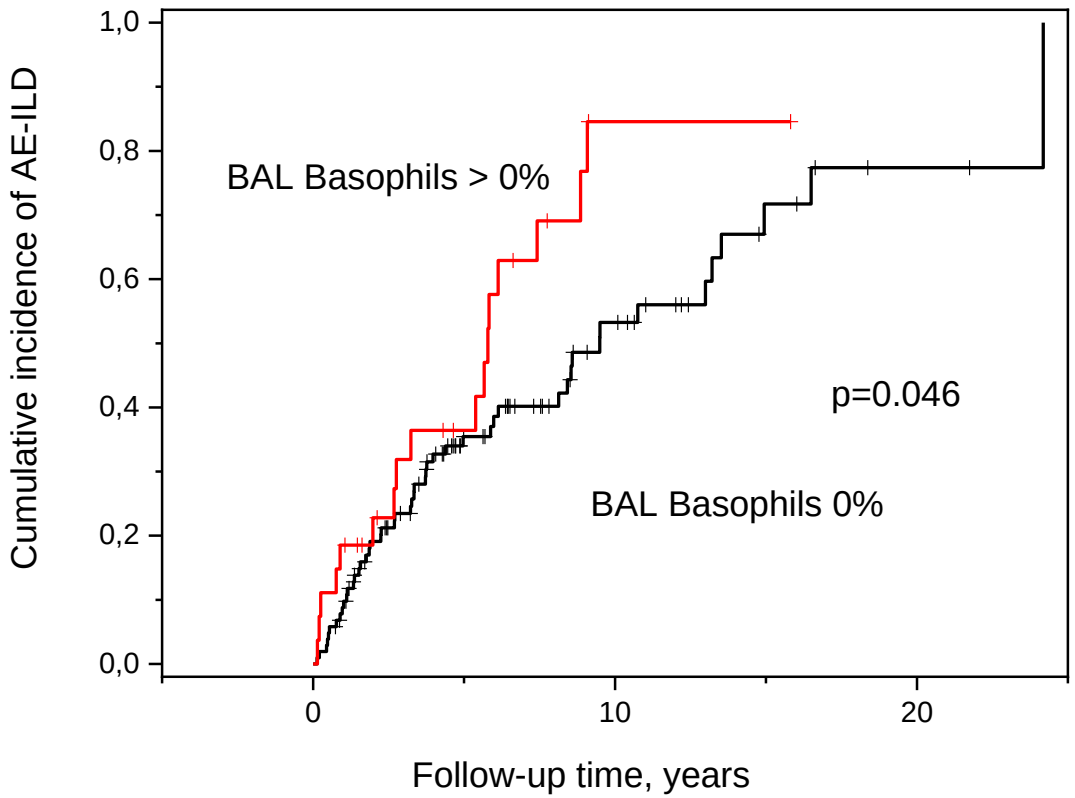
Figure 3. The total cell count (TCC), lymphocytes and neutrophils in the bronchoalveolar lavage (BAL) sampled during an acute exacerbation of an interstitial lung disease were associated with mortality after the BAL procedure date (n=43). A) TCC>160x10^6/L was associated with a more favorable prognosis (TCC>160x10^6/L n=21, TCC≤160x10^6/L n=21, TCC of 1 patient was missing). B) The proportion of lymphocytes in the BAL was associated with a more favorable prognosis (Lymphocytes <20% n=26, ≥20 % n=17). C) The higher proportion of neutrophils in the BAL was associated with a poor prognosis (neutrophils <20 % n=24, ≥20 % n= 19). BAL, bronchoalveolar lavage.


ICD-10 search from medical records:
J84 n=619
J61 n=35
J99 n=0
Total: n=654 hospital treatment periods
1. Previous or concurrent diagnosis of IPF or other fibrosing ILD
2. Acute worsening of respiratory symptoms typically < 1 month duration
Excluded:
No IPF or fibrosing ILD n=82
Patients from other hospital districts with insufficient data for evaluation n=7
Patients with only elective hospitalizations n=50
237 patients (most with multiple hospitalizations)
No BAL
Excluded:
81 patients
BAL
159 patients
Excluded:
Medical treatment initiated for ILD before the first BAL
(3 patients)
1. HRCT with new bilateral ground-glass opacity and/or consolidation superimposed on a fibrotic background pattern (UIP or other) or diffuse alveolar damage in addition to the fibrotic pattern in lung tissue sample
2. Deterioration not fully explained by cardiac failure or fluid overload
23 patients with AE-BAL
20 patients with baseline BAL and AE-BAL
113 therapy naive patients with baseline BAL

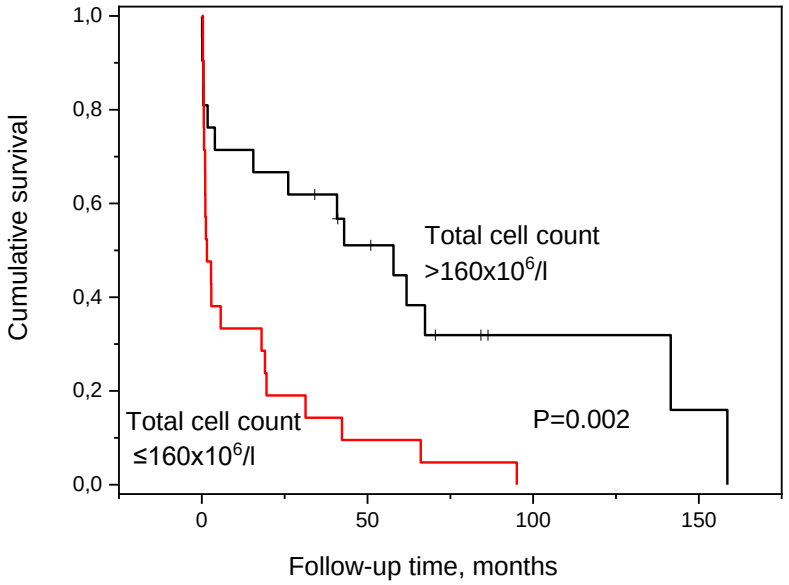
A)


Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
10
20
30
Follow-up time, years
BAL Basophils 0 %
p=0.043
BAL Basophils > 0 %

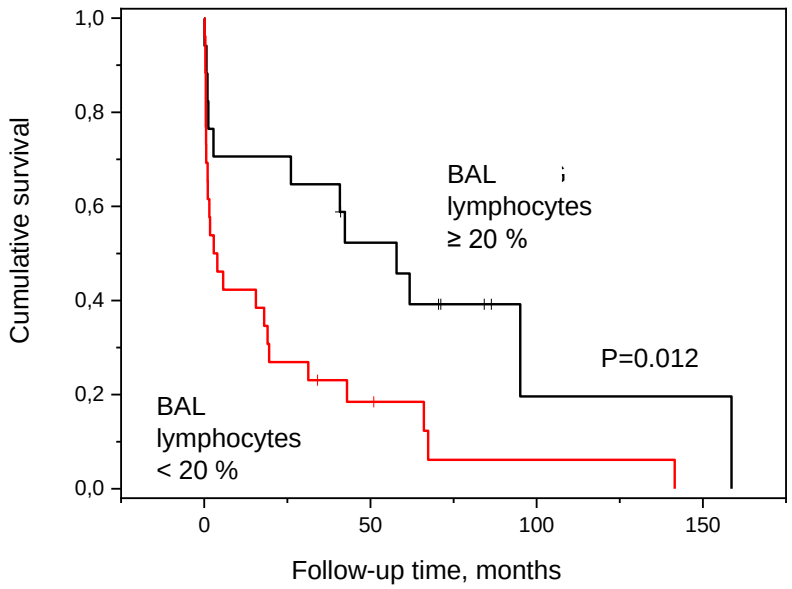
B)


Cumulative incidence of AE-ILD
1,0
0,8
0,6
0,4
0,2
0,0
0
10
20
Follow-up time, years
BAL Basophils > 0%
p=0.046
BAL Basophils 0%

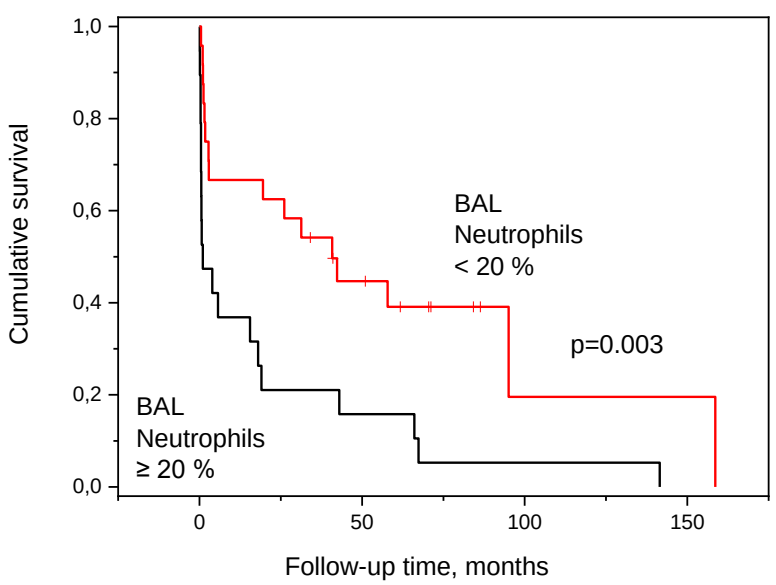
A)


Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
Follow-up time, months
Total cell count
>160x10^6/l
Total cell count
≤160x10^6/l
P=0.002

B)


Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
Follow-up time, months
BAL
lymphocytes
≥ 20 %
P=0.012
BAL
lymphocytes
< 20 %

C)


Cumulative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
50
100
150
Follow-up time, months
BAL
Neutrophils
< 20 %
p=0.003
BAL
Neutrophils
≥ 20 %

Appendix 1.

TABLE A.1 Pharmacological therapy for idiopathic pulmonary fibrosis (IPF) and other fibrosing interstitial lung diseases (ILD) in patients with bronchoalveolar lavage obtained during an acute exacerbation of ILD.

Pharmacological therapy	Total N=43	IPF N=24	Patients with both baseline and AE-BAL data available N=20
Prior to hospital admission due to AE-ILD			
Monotherapy for FILD			
Corticosteroid	7 (16)	4 (17)	5 (25)
Pirfenidone	1 (2.3)	1 (4.2)	1 (5.0)
Nintedanib	0	0	0
Corticosteroid, NAC and azathioprine	7 (16)	5 (21)	6 (30)
Cs for some indication other than ILD	5 (12)	2 (8.3)	2 (10)
Corticosteroid (total)	19 (44)	11 (46)	13 (65)
No medical treatment for ILD	28 (65)	14 (58)	8 (40)
During treatment period			
Corticosteroid at least 20 mg per day[A]	28 (65)	15 (63)	12 (60)
Maximal dose of cs per day (mg) [B]	75 (40–418)	105 (40–500)	135 (64–750)
High-dose intravenous steroids [C]	10 (23)	6 (25)	7 (35)
Antibiotics	37 (86)	21 (88)	20 (100)
Cyclophosphamide	5 (12)	3 (13)	4 (20)
Antimycotics	15 (35)	10 (42)	8 (40)
Antiviral treatment	8 (19)	5 (21)	5 (25)

Data are presented as numbers of patients (%) or median (interquartile range). [A] Dose equivalent for prednisolone. Patients receiving high-dose steroids were excluded. [B] Dose equivalent for prednisolone.[C]500–1000 mg per day for three days, dose equivalent for

prednisolone. AE-BAL, bronchoalveolar lavage performed during AE-ILD; AE-ILD, acute exacerbation of interstitial lung disease; cs, corticosteroid; FILD, fibrosing interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NAC, N-acetylcysteine.

Table A.2 Triggers of AE-ILD and cumulative mortality after the bronchoalveolar lavage (BAL) procedure in patients with acute exacerbation of interstitial lung disease.

	Total N=43	IPF N=24	Other ILD N=19	p-value
Trigger for AE-ILD				
None	31 (72)	18 (75)	13 (68)	0.633
Infection[A]	10 (23)	4 (17)	6 (32)	0.295
Drug[B]	2 (4.7)	2 (8.3)	0	0.495
Cumulative mortality				
30-day mortality	11 (26)	9 (38)	2 (11)	0.077
90-day mortality	19 (42)	14 (58)	4 (21)	0.014
1-year mortality	20 (47)	15 (63)	5 (26)	0.018

Data are presented as numbers of patients (%). [A]Microbiologically or serologically confirmed respiratory infection. [B]One patient had received cytotoxic drugs for breast cancer (fluorouracil, epirubicin and cyclophosphamide) and one other patient had received treatment for lymphoma (rituximab, cyclophosphamide, etoposide and vincristine). IPF, idiopathic pulmonary fibrosis; ILD, interstitial lung disease.

Table A.3 Bronchoalveolar lavage (BAL) cell differential counts at baseline and during an acute exacerbation.

	BAL at baseline				BAL during acute exacerbation of ILD			
BAL	Total N=133	IPF N=81	Other ILD N=52	P-value	Total N=43	IPF N=24	Other ILD N=19	P-Value
Columnar epithelial cells (%)								
PAPA	1.0 (0.0–45.0)[A]	1.0 (0.0–45.0)[B]	0.5 (0.0–18.0)[C]	0.223	2.0 (0.5–45.0)	2.8 (0.0–31.0)	1.0 (0.0–45.0)	0.336

MGG	0.5 (0.0–17.0)[D]	0.5 (0.0–7.5)[A]	0.0 (0.0–17.0)[C]	0.219	0.5 (0.0–53.0)	0.5 (0.0–34.0)	0.5 (0.0–53.0)	0.731
Total cell count (x106/L)	231±178[C]	217±165[C]	252±197	0.266	201±165[C]	197±203	206±100[C]	0.870
Macrophages (%)								
PAPA	67.7±18.1	69.6±16.9	64.9±19.7	0.146	44.3±18.6	45.4±23.1	43.0±20.3	0.722
MGG	70.3±17.7[D]	71.9±16.0[A]	67.9±19.9[C]	0.205	49.5±23.3	48.6±25.1	50.7±21.3	0.765
Lymphocytes (%)								
PAPA	19.7±17.3[C]	15.9±14.1[C]	25.5±20.2	0.004	26.4±18.6	20.8±15.2	33.5±20.5	0.024
MGG	16.6±16.5[D]	12.7±11.6[A]	22.5±20.7[C]	0.003	21.0±16.2	16.3±13.1	26.9±18.1	0.030
Neutrophils (%)								
PAPA	12.6±12.8[C]	14.3±14.2[C]	9.92±9.89	0.039	29.0±23.7	33.7±24.8	23.1±21.4	0.149
MGG	9.1±10.4[D]	10.6±11.7[A]	6.8±7.6[C]	0.028	26.9–24.9	32.0±25.7	20.4±22.1	0.125
Eosinophils (%)								
PAPA[E]	0.0 (0.0–2.0)	0.0 (0.0–2.0)	0.0 (0.0–0.0)	0.069	0.0 (0.0–7.5)	0.0 (0.0–4.0)	0.0 (0.0–7.5)	0.841
MGG	3.7±4.7[D]	4.6±5.3[A]	2.6±3.5[C]	0.004	2.6±4.2	3.1±5.1	1.9±2.7	0.379
Basophils[F] (%)								
MGG	0.0 (0.0–6.5)[B]	0.0 (0.0–6.5)[B]	0.0 (0.0–2.0)	0.777	-	-	-	-

Data is presented as means ± standard deviation and medians (minimum–maximum). P-value was calculated either by using *t*-test or Mann-Whitney U-test, when appropriate. BAL, bronchoalveolar lavage; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; MGG, May-Grünwald-Giemsa; PAPA, Papanicolaou. [A] Data was missing from 3 patients, [B] Data was missing from 2 patients, [C] Data was

missing from 1 patient, [D] Data was missing from 4 patients, [E] Only five IPF patients had evidence of eosinophils with PAPA staining, [F] No basophils could be detected with PAPA staining and basophils could be detected only in 3 AE-BAL samples.

Table A.4 Differential cell counts in bronchoalveolar lavage at baseline and during an acute exacerbation of ILD expressed as numbers of cells per liter.

Cell count (MGG)	BAL at baseline				BAL during acute exacerbation of ILD			
	Total	IPF N=77	Other ILD N=51	p-value	Total N=42	IPF N=24	Other ILD N=18	p-value
Macrophages (E^6/l)	162.2±130.2	163.0±135.9	160.9±122.4	0.928	86.7±61.4	75.7±64.7	101.4±55.2	0.183
Lymphocytes (E^6/l)	45.9±101.6	29.3±49.8	70.7±146.3	0.057	40.3±46.4	24.1±22.6	62.1±60.3	0.019
Neutrophils (E^6/l)	16.9±19.5	18.1±19.8	15.2±19.0	0.411	66±124.6	87.8±157.1	37.4±50.1	0.151
Eosinophils (E^6/l)	8.5±12.9	9.9±13.2	6.5±12.2	0.151	1.3 (0-120.4)	1.3 (0-120.4)	1.7 (0.0-23.5)	0.901
Basophils (E^6/l)[A]	0.0 (0-18.2)	0.0 (0-18.2)	0.0 (0-2.8)	0.761	-	-	-	-

The data is presented as mean±SD or median (min-max). [A]Only three patients with AE-BAL had basophils detected in BAL. BAL, bronchoalveolar lavage; ILD, interstitial lung disease; IPF idiopathic pulmonary fibrosis.