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Conflict of interest statement

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Keywords

Idiopathic pulmonary fibrosis, broncho-alveolar lavage, pulmonary function test, clinical course

Summary

Bronchoalveolar cell count at diagnosis is not linked to lung function decline and exacerbations in a large prospective cohort of idiopathic pulmonary fibrosis patients.

To the Editor,

Broncho-alveolar lavage (BAL) is routinely performed in the diagnostic workup of interstitial lung diseases (ILD). Cell count of leucocytes subsets provides important clues to either confirm or rule out specific diagnosis. The latest guidelines on idiopathic pulmonary fibrosis (IPF) diagnosis include BAL as an important step in the diagnostic process [1,2], either to rule out specific diagnosis or to confirm IPF in the absence of lymphocytosis and/or presence of neutrophilia[2]. Besides, some studies suggest that high BAL eosinophils (>1%) and neutrophils (>10%) counts constitute predictive biomarkers of IPF severity [3,4]. However, this has been poorly prospectively evaluated, especially since the advent of antifibrotics [5]. Furthermore, existing studies have included small populations and yielded conflicting results, notably on the link between BAL eosinophils and mortality [3,4]. Therefore, we investigated the correlation between BAL cell count, longitudinal pulmonary function tests (PFT) and IPF-related death and exacerbations in a multicentric prospective cohort of IPF patients.

The *Prospective Observational Registry to Describe the Disease Course and Outcomes of Idiopathic Pulmonary Fibrosis Patients in a Real-world Clinical Setting (PROOF) in Belgium and Luxembourg* (IPF registry, prolonged by *PROOF-Next*, is a prospective longitudinal and observational study set in eight Belgian centres and one Luxembourg centre . The registry includes patients with an IPF diagnosis according to guidelines.

We selected in the present analysis all patients included in PROOF and PROOF-Next between October 2013 and June 2020 who underwent a BAL for their diagnosis (prior to any IPF treatment) and for whom at least one PFT was available for central analysis. All analysed data were pre-specified in the electronic case report form used for the study. We excluded patients for whom BAL and/or PFT were not available. We collected data on baseline BAL percentage of lymphocytes, eosinophils, neutrophils, and macrophages. All patients were untreated when the BAL was performed.

We analysed possible correlations between cell counts at diagnosis and the variation of force vital capacity (FVC) and lung diffusion capacity (DLCO) in percentage of predicted value from baseline to 6-

and 12-month follow-up. We defined progression as a relative decline of 5% FVC or DLCO. Finally, we studied the potential link between BAL cell composition, transplant-free survival, and exacerbations.

Descriptive statistics are expressed as median and interquartile range unless otherwise specified. Logistic regression models were built to determine whether BAL composition predicts the fact of having a progression as defined by a 12-months relative FVC decline of at least 5% and/or DLCO relative decline of at least 5%. Mann-Whitney Test was used for comparison of non-parametric variables between groups. All statistics were performed by *the Support en Méthodologie et Calcul Statistique (SMCS)*, Université catholique de Louvain. Central and local review boards of participating centres approved the study. All patients provided informed consent.

As of August 4^{th,} 2020, 691 patients were included in the registry. Five hundred seventy-nine patients met our inclusion criteria (444 men, 77%), with a median age of 71 years (IQR 66-76). Most patients (558, 96%) were Caucasian and 450 (78%) were active or former smokers. Median body mass index (BMI) was 27 (IQR 25-30). The main characteristics of the cohort are provided in **table 1**.

Median baseline FVC and DLCO were 82% (IQR 71-97) and 51% (42-61) of expected values, respectively. Median BAL cell composition was 78% (60-88) macrophages, 8% (4-14) lymphocytes, 7% (3-17) neutrophils and 2% (1-5) eosinophils.

Following their workup, 342 patients (59%) were treated with pirfenidone while 136 (23%) received nintedanib. One hundred-one patients (18%) did not receive any antifibrotic treatment.

At six-month follow-up (median 205 days, IQR 173-237), PFT data were available for 307 patients. Median FVC and DLCO were 81% (IQR 69-97) and 48% (39-61).

At twelve-month follow-up (median 351 days, 328-384), data were available for 409 patients. Median FVC and DLCO were 81% (68-96) and 49% (39-59). Twelve months FVC relative decline was -1.8% (-8.3 - +6.5) and DLCO relative decline was -6.1% (-17.6 - +6.2).

We used a model of binary logistic regression to predict the effect of BAL lymphocytosis, neutrophils and eosinophils on one-year progression, exacerbations and a composite endopoint combining transplant-free survival and exacerbations. Baseline FVC, DLCO, sex and age were used as control variables. As shown in table 2, none of the BAL cell subtypes count was related to outcomes of interest, apart from a trend (OR 0.9, P=0.09) for the effect of lymphocytosis on the composite endpoint (**table 2**).

We also analysed whether BAL cell count could discriminate between *rapid progressors*, displaying a 12-month relative decline of FVC >10% and/or DLCO > 15% (non-missing data N=177) and *non-progressors* (non-missing data N=123). The comparison of BAL cell composition between these two groups did not show any significant difference (P=0.34).

Similarly, we compared *exacerbators* and *non-exacerbators*. Sixty-seven acute exacerbations occurred in 59 patients during a median follow-up of 462 days (211-699). When comparing exacerbators (N=59)

and non-exacerbators (N=520), there were no significant differences regarding BAL cell differential counts at baseline (P=0.11, Mann-Whitney *U* test).

During follow-up, 93 patients (16%) died from an IPF-related condition and 12 patients were transplanted. Transplant-free survivors (N=486) had a significantly higher median lymphocyte count in their BAL as compared to non-survivors and lung transplant recipients (8% (4-15) vs 6% (3-13), P=0.04, Mann Whitney *U* test). When comparing 105 patients who died in relation with IPF or had acute exacerbations to the others (N=474), a significant difference in lymphocytosis was also found (6% (3-13) vs 8% (4-15), Mann-Whitney U test, P=0.02).

In conclusion, in a large prospective IPF cohort, in the era of antifibrotic drugs, BAL cell count at diagnosis was not related to baseline PFT and was not predictive of lung function decline. Patients who died for an IPF-related condition or were transplanted displayed modestly lower lymphocyte counts in BAL. This latter finding might suggest the existence of different BAL-related phenotypes among IPF patients, but this needs further validation and confirmation in other cohorts. Especially, lymphocytes could not be considered as a biomarker given the large overlap between lymphocytes counts in subpopulations.

Altogether, our observations question the usefulness of BAL as a predictive tool for IPF severity if one relies on differential cell counts only. However, several recent reports illustrate the use of other BAL biomarkers: mRNA expression of BAL fluid is able to discriminate IPF from sarcoidosis [6], BAL concentration of matrix metalloprotease 10 (MMP10) is correlated to disease severity and progression [7], and the “rapid progressors” patients elicit higher levels of MMP-9 transcripts in their BAL [8]. Several other BAL biomarkers, usually linked to extracellular matrix turnover, immune dysregulation and epithelial cells dysfunction are currently under investigation ([9]). Finally, there are also attempts to develop predictive IPF BAL gene signatures[10,11] albeit these require validation in large, multi-ethnic populations.

Despite being prospective and multicentric, our study has some limitations: firstly, as more than 80% of patients were treated with antifibrotics, we cannot certify that our results apply to an untreated population. Secondly, the real-life settings of this study implied a certain loss of data after 6 and 12-month follow-up, as regular patient follow-up did not always correspond to the 6- and 12-months interval we determined. Finally, although most patients were handled in experienced ILD centres, small differences in patients’ management may affect the results in this multi-centric study.

In conclusion, although useful for diagnostic purposes, BAL cell count was poorly linked to outcome in IPF. Future and ongoing research might lead to the use of BAL theragnostic biomarkers.

References

1. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med.* 2018;198(5):e44-e68.
2. Raghu G, Remy-Jardin M, Richeldi L, 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;205(9):e18-e47.
3. Peterson MW, Monick M, Hunninghake GW. Prognostic role of eosinophils in pulmonary fibrosis. *Chest.* 1987;92(1):51-56.
4. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE, Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest.* 2008;133(1):226-232.
5. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. *Lancet.* 2017.
6. Paplinska-Goryca M, Goryca K, Misiukiewicz-Stepien P, et al. mRNA expression profile of bronchoalveolar lavage fluid cells from patients with idiopathic pulmonary fibrosis and sarcoidosis. *Eur J Clin Invest.* 2019;49(9):e13153.
7. Sokai A, Handa T, Tanizawa K, et al. Matrix metalloproteinase-10: a novel biomarker for idiopathic pulmonary fibrosis. *Respir Res.* 2015;16:120.
8. Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. *PLoS One.* 2007;2(5):e482.
9. Inoue Y, Kaner RJ, Guiot J, et al. Diagnostic and Prognostic Biomarkers for Chronic Fibrosing Interstitial Lung Diseases With a Progressive Phenotype. *Chest.* 2020;158(2):646-659.
10. Xia Y, Lei C, Yang D, Luo H. Construction and validation of a bronchoalveolar lavage cell-associated gene signature for prognosis prediction in idiopathic pulmonary fibrosis. *Int Immunopharmacol.* 2021;92:107369.
11. De Sadeleer LJ, Verleden SE, Schupp JC, et al. BAL Transcriptomes Characterize Idiopathic Pulmonary Fibrosis Endotypes With Prognostic Impact. *Chest.* 2022.

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165 **Table 1**

Baseline characteristics of the cohort (N=579)	
Demographics	
Gender (M/F, %)	444/135 (77/23)
Age (median, IQR)	71 (66-76)
Ethnicity (N, %) • Caucasian • Other	 558 (96%) 21 (4%)
Former or current smoker (N, %)	450 (78%)
Familial history of IPF (N, %)	63 (11%)
IPF diagnosis: lung biopsy (N=186 biopsies for 178 patients, 31%)	
Surgical biopsy (open or VATS)	116 (20%)
Transbronchial (cryo)biopsies	59 (10%)
Not specified	11 (2%)
IPF diagnosis certainty on histology (N, % of biopsies)	
Definite UIP	101 (54%)
Probable UIP	47 (25%)
Possible UIP	27 (5%)
Not contributive	11 (2%)
Bronchoalveolar lavage cell count (%, median, IQR)	
Monocytes/macrophages (computed from other data)	78 (60-88)
Lymphocytes	8 (4-14)
Neutrophils	7 (3-17)
Eosinophils	2 (1-5)
Baseline pulmonary function tests	
FVC (%, median, IQR)	82 (71-97)
DLCO (%, median, IQR)	51 (42-61)

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Table 2: predictive effect of variables in a binary logistic regression model*

Outcome	BAL cell count	OR (CI 95)	P-value
12-month progression§	Lymphocytes	0.98 (0.96-1.01)	0.23
	Neutrophils	1.00 (0.99-1.02)	0.67
	Eosinophils	1.01 (0.96-1.06)	0.72
Exacerbations	Lymphocytes	1.01 (0.97-1.03)	0.74
	Neutrophils	1.01 (0.99-1.03)	0.09
	Eosinophils	0.98 (0.90-1.05)	0.63
Death/transplantation and/or exacerbations	Lymphocytes	0.97 (0.93-1.00)	0.08
	Neutrophils	1.00 (0.98-1.02)	0.89
	Eosinophils	1.01 0.94-1.07)	0.76

*: baseline forced vital capacity (FVC), baseline lung diffusion capacity, sex and age are used as control variables

§: 12-month relative FVC decline ≥5%

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Abbreviation list (in order of appearance in the text)

BAL	bronchoalveolar lavage
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis
PFT	pulmonary function tests
FVC	forced vital capacity
DLCO	lung diffusion capacity
IQR	interquartile range
BMI	body-mass index
MMP10	matrix metalloproteinase 10