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Scientific Letter

Peripheral Immune Cell Profiling Reveals Distinct Immune Hallmarks in Progressive Pulmonary Fibrosis

To the Director,

Within the spectrum of fibrosing interstitial lung diseases (f-ILDs) there is a subset of patients who exhibit a similar clinical course and prognosis to those with idiopathic pulmonary fibrosis (IPF), named progressive pulmonary fibrosis (PPF).^{1,2} The mechanisms underlying PPF are unclear. We hypothesize that the peripheral immune cell profile in PPF may differ from non-progressive f-ILDs (non-PPF). To test this hypothesis, we determined the blood immune cell profile in patients with f-ILDs at diagnosis and we explored their association with disease severity, as determined by lung function (forced vital capacity [FVC] and diffusing capacity for carbon monoxide [DL_{CO}]. Patients were then followed-up for 12 months and classified *post hoc* as PPF or non-PPF. This allowed us to compare the relationship of the immune cell profile determined at baseline and PPF.

This observational study included 33 patients with f-ILDs, other than IPF. Diagnosis of ILDs was established through a multidisciplinary discussion based on clinical characteristics along with HRCT scan, bronchoalveolar lavage, and lung biopsy patterns if appropriate. Disease progression was defined as per international guidelines (ATS/ERS/JRS/ALAT), by ≥ 2 of the following criteria: worsening respiratory symptoms, lung function progression (absolute change in FVC \geq 5% and/or DL_{CO} \geq 10%) and/or radiological progression.¹ Lung function was measured according to international standards. Reference values were those of Roca et al.³ Peripheral blood was collected at diagnosis in EDTA tubes. Fluorescence activated cell staining and analysis (FACS) was used to profile B cells, T cells (and subpopulations), NK cells, NKT-like cells, monocytes, neutrophils, and eosinophils. Briefly, $120 \,\mu l$ of blood was incubated with $30 \,\mu l$ of the antibody mix during 30 min at 4 °C. Then, erythrocytes were lysed (BD FACS Lysing Solution, USA), and cells were incubated with the fixable Viability Stain (440UV, BD 566332) 15 min at room temperature in the dark, washed and fixed using PFA 4%. Fixed samples data were acquired with a BD LSRFortessa 5 laser flow cytometer. FlowJo version 10 software (FlowJo LL, USA) was used for the analysis. The Ethics Committee of our institution approved the study (HCB/2017/0901), and all participants signed their informed consent. All statistics were computed with R version 3.6.2, using custom scripts.

Eleven patients (33.3%) exhibited PPF during follow-up, whereas 22 (66.7%) remained stable. Table 1 presents the main demographic and clinical characteristics of these two groups at diagnosis, with further stratification by non-idiopathic pulmonary fibrosis subtypes available in Table S1. Age, gender, smoking status and proportion of f-ILD subtypes were similar across main groups. Most patients were naïve to immunosuppressive and antifibrotic treatment. In the entire population studied (n = 33), we found significant correlations between several blood immune markers and lung function (Fig. 1A): FVC was negatively correlated with CD8⁺HLA-DR⁺ T cells and central memory (CM) CD4 T cells, whereas both FVC and DL_{CO} were negatively related with the percentage of T helper type 1 (Th1) cells and CD4⁺CD28⁻HLA-DR⁺. We then explored which markers at diagnosis were associated with PPF. We found that PPF patients showed a significantly higher percentage of NKT-like cells (Fig. 1B and C), and a significant negative correlation between FVC and the percentage of CD8 T cells, which was not observed in non-PPF patients (Fig. 1D).

The main and novel observations of this study are that: (1) in the entire population of f-ILDs, several CD8 and CD4 subpopulations correlate with lung function severity at the time of diagnosis and (2) NKT-like cells levels at diagnosis are associated with PPF.

We found several immunological changes regarding CD8 and CD4 T cell repertoire that correlated with lung function severity at the time of diagnosis in the whole population of f-ILDs (Fig. 1A) including that CD8⁺HLA-DR⁺ T cells and CM CD4 T cells negatively correlated with FVC, and CD4⁺CD28⁻HLA-DR⁺ T cells negatively correlated with FVC and DL_{CO}. These observations suggest that an exhausted immune system may have a role in the pathogenesis of f-ILDs because elderly persons also show reduced levels of naïve T cells, a relative increase of memory T lymphocytes, and accumulation of antigen experienced CD4⁺CD28⁻ T cells.^{4,5} In fact, in keeping with these observations, previous studies also showed that increased levels of CD4+CD28- T cells (also a marker of immune exhaustion) are associated with poor outcomes in IPF patients.⁶ Finally, we observed a negative correlation between Th1 cells and both FVC and DL_{CO} at diagnosis, likely indicating a pro-fibrotic role of Th1 cells.

A major question in this analysis was to investigate if any immune marker determined at diagnosis in a population of f-ILDs at large was associated with PPF. We found that NKT-like cells were increased at baseline in patients who eventually developed PPF as compared to those with non-PPF (Fig. 1B). A previous report showed increased NKT-like cells in bronchoalveolar lavage of f-ILDs.⁸ Here, we extend these previous observations to circulating blood. NKT-like cells provide protection against pathogens and tumor cells but, of note here, may also be related to cell senescence.⁹ Additionally, while previous literature suggests a potential influence of smoking on cell populations, the statistical power of the present study was insufficient to convincingly demonstrate the interaction between NK T-like cells decrease and smoking status.

Interestingly, a senescent T-cell phenotype has also been reported in IPF patients.¹⁰ Age-related changes in T-cell reservoirs and function includes an expansion of mostly highly differentiated CD8⁺ T cells, but its role in PPF is unknown. We found that

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Table 1

Characteristics of Participants and Immune Populations at Diagnosis in Fibrosing ILD Patients With and Without PPF During Follow-up.

Characteristics No.PP (r.e.2) PP (r.e.1) p-value Included patients 7.2.9(9.12) 0.171 Gender, n(k) 7.2.9(9.12) 0.171 Gender, n(k) 7.2.9(9.12) 0.171 Gender, n(k) 7.2.9(9.12) 0.171 Gender, n(k) 7.3.681 0.171 Formales 13 (59.13) 3.2733 Smoking history at baseline, n(k) 7.63.681 0.111 Never-smokers 5.22.73 7.63.681 0.111 Never-smokers 7.33 (170.) 6.62.(13.41) 0.763 Smoking history at baseline, n(x) 7.91.1 (18.2) 6.62.(13.41) 0.025 Pulmonary function at diagnosis, men (+5D) 4.18.22.1 3.12.23.1 0.025 Dist, Spredicted 7.91.1 (18.23) 6.62.(13.41) 0.025 Dist, Spredicted 7.93.1 (18.23) 3.12.23.1 0.005 IP 4.19.23.1 3.12.23.1 0.005 IP 4.19.23.1 3.12.23.1 1.000 Marine facted-LLD 8.13.64.31 3.12.23.1 1.00				
<table-container>Hereiter729(19)729(19)729(19)729(19)729(19)Center, normal segment segm</table-container>	Characteristics	Non-PPF ($n = 22$)	PPF(n=11)	<i>p</i> -Value
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Smoking related-LD 8 (36.4%) 3 (27.3%) Immuosuppresant use at diagnosis, n (%) Immuosuppresant use at diagnosis, n (%) 1.000 Methotrexate 1 (4.55%) 0 (0%) 1.000 Mycophenolate 1 (4.55%) 0 (0%) 1.000 Mycophenolate 1 (4.55%) 2 (18.2%) 0.252 Prednisone 5 (22.7%) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 0.252 Prednisone 5 (22.7%) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 1.000 Site immune cells, mean (±SD) or median [IQR] 29.5 (10.1) 28.4 (10.0) 0.778 Lymphocytes CD3* 29.5 (10.1) 28.4 (10.0) 0.468 CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* T cells 25.1 (24.16.16) 45.1 (24.0) 0.468 CD8* T cells 25.6 [20.1] 27.4 (16.8) 0.609 Effector CD8* T cells 35.6 [20.1] 27.4 (16.8) 0.39 Memony central CD8* T cells 35.6 [20.1]	Fibrosing HP	6 (27.3%)	3 (27.3%)	
Immunosuppressant use at diagnosis, n (%) 1 (4.55%) 0 (0%) 1.000 Leflunomide 1 (4.55%) 0 (0%) 1.000 Mycophenolate 1 (4.55%) 2 (18.2%) 0.252 Prednisone 1 (4.55%) 2 (18.2%) 0.000 Rituximab 0 (0%) 2 (18.2%) 1.000 Difference 20.5 (10.1) 28.4 (10.0) 0.778 Signed Calls 338 (15.0) 29.1 (10.5) 0.299 CD8* T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8* T cells 51.4 (21.6) 45.1 (24.0) 0.468 DB* HA-DR* T cells 51.4 (21.6) 45.1 (24.0) 0.468 DB* HA-DR* T cells 51.0 (2.0.1;63.4] 27.0 [21.2;53.8] 0.760 Memory central CD8* T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory effector CD8* T cells 55.7 (20.0) 37.3 (23.2) 0.832 Odd* CD28* T cells 55.7 (20.0) 37.3 (23.2) 0.832 Odd* CD28* T cells 0.618 (35.62.60] 31.9 [13.5;40.8] 0.606	Smoking related-ILD	8 (36.4%)	3 (27.3%)	
Methotrizate 1 (4.55%) 0 (0%) 1.000 Leflunomide 1 (4.55%) 0 (0%) 1.000 Mycophenolate 1 (4.55%) 2 (18.2%) 0.252 Prednisone 5 (22.7%) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 0.252 Prednisone 5 (22.7%) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 0.000 Immune cells, mean (±SD) or median [IQR] 1 29.5 (10.1) 28.4 (10.0) 0.778 CD8* T cells 338 (15.0) 29.1 (10.5) 0.299 0.468 CD8*T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8*T cells 51.5 (20.0) 37.3 (23.2.8) 0.359 Memory central CD8*T cells 8.36 [45.115.2] 1.10 [6.38:20.8] 0.359 Memory central CD8*T cells 5.9 (714.3) 61.1 (14.8) 0.796 CD4*T cells 5.9 (714.3) 61.1 (14.8) 0.796 CD4*T cells 0.3 [7.82;14.0) 7.87 (5.45;15.8] 0.401 Effector C	Immunosuppressant use at diagnosis. n (%)			
Leflunomide 1 (4.55%) 0 (0%) 1.000 Mycophenolate 1 (4.55%) 2 (18.2%) 0.252 Prednisone 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 1.000 Immune populations 2 (18.2%) 1.000 Adaptive immune cells, mean (±5D) or median [IQR] 29.5 (10.1) 28.4 (10.0) 0.778 Lymphocytes CD3* 29.5 (10.1) 28.4 (10.0) 0.778 CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8* TLAPK*T cells 51.4 (21.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.1/63.4] 27.0 [21.2:53.8] 0.760 Memory central CD8* T cells 35.5 (20.0) 37.3 (23.2) 0.832 CD4* T cells 59.7 (14.3) 61.1 (14.8) 0.796 CD4* T cells 10.6 [3.6:26.0] 31.9 [13.5:40.8] 0.620 CD4* T cells 10.3 [7.82;1.40] 7.8 [5.4:51.8] 0.401 Effector CD4* T cells 0.3 [7.82;1.40] 7.8 [5.4:51.8] 0.401 Effector CD4* T cells <td>Methotrexate</td> <td>1 (4.55%)</td> <td>0 (0%)</td> <td>1.000</td>	Methotrexate	1 (4.55%)	0 (0%)	1.000
Mycophenolate 1 (4.552) 2 (18.28) 0.252 Prednisone 5 (22.78) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.28) 1.000 Immue populations 2 (18.28) 1.000 Adaptive immune cells, mean (±SD) or median [IQR] 29.5 (10.1) 28.4 (10.0) 0.778 CD8' T cells 33.8 (15.0) 29.1 (10.5) 0.299 D8' T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8' T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8' T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8' T cells 51.4 (21.6) 27.4 (16.8) 0.609 Effector CD8' T cells 35.0 [20.1;63.4] 27.0 (21.2;53.8] 0.760 Memory effector CD8' T cells 8.36 [4.51;15.2] 11.0 [6.38;20.8] 0.339 Memory effector CD8' T cells 5.5 (20.0) 37.3 (23.2) 0.832 CD4' T cells 1.6 [8.36;26.0] 3.19 [13.5;40.8] 0.620 CD4' T cells 1.6 [8.36;26.0] 3.19 [13.5;40.8] 0.401 Effe	Leflunomide	1 (4.55%)	0 (0%)	1.000
Prednisone 5 (22.7%) 3 (27.3%) 1.000 Rituximab 0 (0%) 2 (18.2%) 1.000 Immure populations 2 2 2 2 2 1.000 Adaptive immure cells, mean (±SD) or median [IQR] 2 2.9.5 (10.1) 28.4 (10.0) 0.778 CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8*HLA-DR* T cells 24.4 (12.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory effector CD8* T cells 35.5 (20.0) 37.3 (23.2) 0.832 CD4* T cells 55.7 (14.3) 61.1 (14.8) 0.796 CD4* CD28* T cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.620 CD4* CD28* T cells 1.00 [8.3c;26.0] 31.9 [13.5;40.8] 0.056 CD4* CD28* T cells 1.03 [7.82;14.0] 7.87 [5.8;15.8] 0.401 Effector CD4* T cells 0.8 [0.20;3.34] 2.27 [0.5;5.66] 0.349 Memory centru	Mycophenolate	1 (4.55%)	2 (18.2%)	0.252
Rituximab 0 (0%) 2 (18.2%) 1.000 Immune populations Adaptive immune cells, mean (±SD) or median [IQR]	Prednisone	5 (22.7%)	3 (27.3%)	1.000
Immune populations Adaptive immune cells, mean (±SD) or median [IQR] Lymphocytes (D3* 29.5 (10.1) 28.4 (10.0) 0.778 DS* T Cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* T Cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8* T Cells 51.4 (21.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory central CD8* T cells 83.6 (451;15.2] 11.0 [6.38;20.8] 0.359 Memory effector CD8* T cells 35.5 (20.0) 37.3 (23.2) 0.832 CD4* T cells 59.7 (14.3) 61.1 (14.8) 0.796 CD4* T cells 16.0 [8.66;26.0] 31.9 [13.5;40.8] 0.650 CD4* T cells 16.0 [8.66;26.0] 31.9 [13.5;40.8] 0.401 Effector CD4* T cells 0.88 [0.20;33.4] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349	Rituximab	0 (0%)	2 (18.2%)	1.000
Adaptive immune cells, mean (±SD) or median [IQR] 29.5 (10.1) 28.4 (10.0) 0.778 Lymphocytes CD3* 29.5 (10.1) 28.4 (10.0) 0.778 CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8* T cells 24.4 (12.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.163.4] 27.0 [21.2;53.8] 0.760 Memory central CD8* T cells 35.5 (20.0) 37.3 (23.2) 0.832 Memory central CD8* T cells 55.7 (14.3) 61.1 (14.8) 0.796 CD4* CD28 T cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.650 CD4* CD28 T cells 10.0 [8.36;26.0] 31.9 [13.5;40.8] 0.056 CD4* CD28 T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4* T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.3349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 10.0 (5.20) 6.6 (5.48) 0.01	Immune populations			
Lymphocytes CD3* 29.5 (10.1) 28.4 (10.0) 0.778 CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8*CD28 - T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8*HLA-DR*T cells 24.4 (12.5) 27.4 (16.8) 0.609 Effector CD8*T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory central CD8*T cells 8.36 [451;15.2] 11.0 [6.38;20.8] 0.359 Memory effector CD8*T cells 8.36 [451;15.2] 11.0 [6.38;20.8] 0.359 CD4*T cells 55.7 (14.3) 61.1 (14.8) 0.796 CD4*T Cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.620 CD4*t CD28 - T cells 10.0 [8.36;26.0] 31.9 [13.5;40.8] 0.056 CD4*t CD28 - T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4*T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory central CD4*T cells 2.56 (17.0) 47.5 (13.1) 0.730 Memory effector CD4*T cells 2.56 (17.0) 47.5 (13.1) 0.730 Memory effector CD4*T cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 <tr< td=""><td>Adaptive immune cells, mean $(\pm SD)$ or median [IQR]</td><td></td><td></td><td></td></tr<>	Adaptive immune cells, mean $(\pm SD)$ or median [IQR]			
CD8* T cells 33.8 (15.0) 29.1 (10.5) 0.299 CD8* CD28 - T cells 51.4 (21.6) 45.1 (24.0) 0.468 CD8*HLA-DR* T cells 24.4 (12.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory central CD8*T cells 8.36 [451;15.2] 11.0 [6.38:20.8] 0.359 Memory effector CD8*T cells 8.36 [451;15.2] 11.0 [6.38:20.8] 0.359 Memory effector CD8*T cells 55.7 (14.3) 61.1 (14.8) 0.796 CD4* T cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.620 CD4*CD28 - T cells 10.0 [8.36;26.0] 31.9 [13.5;40.8] 0.056 CD4*CD28 - T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4*T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory central CD4*T cells 45.6 (17.0) 47.5 (13.1) 0.730 Memory effector CD4*T cells 23.0 (12.4) 22.1 (13.0) 0.846 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 24.0 (2.6) 6.60 (1.17) 0.196	Lymphocytes CD3 ⁺	29.5 (10.1)	28.4 (10.0)	0.778
CD8*CD28 - T cells51.4 (21.6)45.1 (24.0)0.468CD8*HLA-DR*T cells24.4 (12.5)27.4 (16.8)0.609Effector CD8*T cells35.0 [20.1;63.4]27.0 [21.2;53.8]0.760Memory entral CD8*T cells8.36 [4.51;15.2]11.0 [6.38;20.8]0.359Memory effector CD8*T cells35.5 (20.0)37.3 (23.2)0.832CD4*T cells59.7 (14.3)61.1 (14.8)0.796CD4*CD28 - T cells4.75 [0.63;10.9]7.29 [2.02;10.8]0.626CD4*CD28 - T cells16.0 [8.36;26.0]31.9 [13.5;40.8]0.056CD4*CD28 - T cells10.3 [7.82;14.0]7.87 [5.48;15.8]0.401Effector CD4* T cells0.88 [0.20;3.34]2.27 [0.52;5.66]0.349Memory entral CD4*T cells23.0 (12.4)22.1 (13.0)0.846Th1 cells18.4 [13.5;24.2]19.8 [15.9;31.5]0.468B cells10.0 (5.20)6.67 (5.48)0.107Innate immune cells, mean (±SD) or median [IQR]CD1* monocytes7.34 (2.06)6.60 (1.17)0.196Fosinophils0.89 [0.27;1.33]0.73 [0.61;0.80]1.000NK cells10.0 [5.72;13.4]7.70 [5.29;17.2]0.939NKT-like cells1.24 [0.98;1.96]2.34 [1.29;4.02]<0.05	CD8 ⁺ T cells	33.8 (15.0)	29.1 (10.5)	0.299
CD8*HLA-DR* T cells 24.4 (12.5) 27.4 (16.8) 0.609 Effector CD8* T cells 35.0 [20.1;63.4] 27.0 [21.2;53.8] 0.760 Memory central CD8* T cells 8.36 [4.51;15.2] 11.0 [63:8:20.8] 0.359 Memory effector CD8* T cells 35.5 (20.0) 37.3 (23.2) 0.832 CD4* T cells 59.7 (14.3) 61.1 (14.8) 0.796 CD4* CD28 - T cells 59.7 (14.3) 61.1 (14.8) 0.056 CD4* CD28 - T cells 16.0 [8.36;26.0] 31.9 [13.5;40.8] 0.056 CD4* CD28 - HLA-DR* T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory central CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 23.0 (12.4) 2.1 (13.0) 0.846 Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 (0.61:0.80] 1.00	CD8 ⁺ CD28 ⁻ T cells	51.4 (21.6)	45.1 (24.0)	0.468
Effector CD8* T cells35.0 [20.1;63.4]27.0 [21.2;53.8]0.760Memory central CD8* T cells8.36 [4.51;15.2]11.0 [6.38;20.8]0.359Memory effector CD8* T cells35.5 (20.0)37.3 (23.2)0.832CD4* T cells59.7 (14.3)61.1 (14.8)0.796CD4* CD28 - T cells4.75 [0.63;10.9]7.29 [2.02;10.8]0.620CD4* CD28 - T cells16.0 [8.36;26.0]31.9 [13.5;40.8]0.056CD4* CD28 - HLA-DR* T cells10.3 [7.82;14.0]7.87 [5.48;15.8]0.401Effector CD4* T cells0.88 [0.20;3.34]2.27 [0.52;5.66]0.349Memory central CD4* T cells45.6 (17.0)47.5 (13.1)0.730Memory effector CD4* T cells23.0 (12.4)22.1 (13.0)0.846Th1 cells18.4 [13.5;24.2]19.8 [15.9;31.5]0.468B cells10.0 (5.20)6.67 (5.48)0.107Innate immune cells, mean (±SD) or median [IQR]CD14* monocytes6.60 (1.17)0.196CD14* monocytes6.18 (9.13)61.9 (11.6)0.979NK cells10.0 [5.72;13.4]7.70 [5.29;17.2]0.939NK cells10.0 [5.72;13.4]7.70 [5.29;17.2]0.939	CD8 ⁺ HLA-DR ⁺ T cells	24.4 (12.5)	27.4 (16.8)	0.609
Memory central CD8' T cells8.36 [4.51;15.2]11.0 [6.38;20.8]0.359Memory effector CD8 T cells35.5 (20.0)37.3 (23.2)0.832CD4' T cells59.7 (14.3)61.1 (14.8)0.796CD4'CD28 - T cells4.75 [0.63;10.9]7.29 [2.02;10.8]0.620CD4'CD28 - HLA-DR T cells16.0 [8.36;26.0]31.9 [13.5;40.8]0.056CD4'T C28 - HLA-DR T cells10.3 [7.82;14.0]7.87 [5.48;15.8]0.401Effector CD4' T cells0.88 [0.20;3.34]2.27 [0.52;5.66]0.349Memory central CD4' T cells18.4 [13.5;24.2]19.8 [15.9;31.5]0.468B cells10.0 (5.20)6.67 (5.48)0.107Innate immune cells, mean (±SD) or median [IQR]UUUUCD14' monocytes7.34 (2.06)6.60 (1.17)0.196Eosinophils0.89 [0.27;1.33]0.73 [0.61;0.80]1.000Neutrophils61.8 (9.13)61.9 (11.6)0.979NK cells10.0 [5.72;13.4]7.70 [5.29;17.2]0.939NK cells1.24 [0.98;1.96]2.34 [1.29;4.02] 4.005	Effector CD8 ⁺ T cells	35.0 [20.1;63.4]	27.0 [21.2;53.8]	0.760
Memory effector CD8* 1 cells 35.5 (20.0) 37.3 (23.2) 0.832 CD4* T cells 59.7 (14.3) 61.1 (14.8) 0.796 CD4*CD28- T cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.620 CD4*CD28-T cells 16.0 [8.36;26.0] 31.9 [13.5;40.8] 0.056 CD4*LD28-T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory effector CD4* T cells 23.0 (12.4) 22.1 (13.0) 0.846 Memory effector CD4* T cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] 40.5 <	Memory central CD8 ⁺ T cells	8.36 [4.51;15.2]	11.0 [6.38;20.8]	0.359
CD4' T cells 59,7 (14.3) 61.1 (14.8) 0.796 CD4' CD28 - T cells 4.75 [0.63;10.9] 7.29 [2.02;10.8] 0.620 CD4'CD28 - HLA-DR* T cells 16.0 [8.36;26.0] 31.9 [2.13;40.8] 0.056 CD4'TCD28 - HLA-DR* T cells 10.3 [7.82;14.0] 7.87 [5.48;15.8] 0.401 Effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory central CD4* T cells 45.6 (17.0) 47.5 (13.1) 0.730 Memory effector CD4* T cells 23.0 (12.4) 22.1 (13.0) 0.846 Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Memory effector CD8 ⁺ T cells	35.5 (20.0)	37.3 (23.2)	0.832
CD4*CD28*Teells4.75 [0.63;10.9]7.29 [2.02;10.8]0.620CD4*CD28*HLA-DR* T cells160 [8.36;26.0]31.9 [13.5;40.8]0.056CD4*HLA-DR* T cells10.3 [7.82;14.0]7.87 [5.48;15.8]0.401Effector CD4* T cells0.88 [0.20;3.34]2.27 [0.52;5.66]0.349Memory central CD4* T cells45.6 (17.0)47.5 (13.1)0.730Memory effector CD4* T cells23.0 (12.4)22.1 (13.0)0.846Th1 cells18.4 [13.5;24.2]19.8 [15.9;31.5]0.468B cells10.0 (5.20)6.67 (5.48)0.107Innate immune cells, mean (±SD) or median [IQR]7.34 (2.06)6.60 (1.17)0.196CD14* monocytes7.34 (2.06)6.60 (1.17)0.196Eosinophils0.89 [0.27;1.33]0.73 [0.61;0.80]1.000Neutrophils61.8 (9.13)61.9 (11.6)0.979NK cells1.04 [0.98;1.96]2.34 [1.29;4.02]<0.05	CD4' I cells	59.7 (14.3)	61.1 (14.8)	0.796
CD4 CD2s HLA-DK Teells 160 [0.56,260] 51.9 [15.3,40.8] 0.056 CD4 HLA-DK T Cells 10.3 [7.82;14.0] 7.87 [548;15.8] 0.401 Effector CD4* T cells 0.88 [0.20;3.34] 2.27 [0.52;5.66] 0.349 Memory central CD4* T cells 45.6 (17.0) 47.5 (13.1) 0.730 Memory effector CD4* T cells 23.0 (12.4) 22.1 (13.0) 0.846 Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 2.734 (2.06) 6.60 (1.17) 0.196 CD14* monocytes 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] 4.05	$CD4^{+}CD28^{-}$ I cells	4.75 [0.63;10.9]	7.29 [2.02;10.8]	0.620
Edit Inde-Drift Teells 10.5 [7.62, (4.0] 7.87 [5.48, (1.53] 0.401 Effector CD4' Teells 0.88 [0.20; 3.34] 2.27 [0.52; 5.66] 0.349 Memory central CD4' T cells 45.6 (17.0) 47.5 (13.1) 0.730 Memory effector CD4' T cells 23.0 (12.4) 22.1 (13.0) 0.846 Th1 cells 18.4 [13.5; 24.2] 19.8 [15.9; 31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 2 2 10.0 (5.20) 10.0 (5.00) CD14* monocytes 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27; 1.33] 0.73 [0.61; 0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72; 13.4] 7.70 [5.29; 17.2] 0.939 NKT-like cells 1.24 [0.98; 1.96] 2.34 [1.29; 4.02] <0.05	CD4 $CD20$ $ELA-DK$ I Cells	10.0 [8.50,20.0]	51.9 [15.5,40.6] 7 97 [5 49.15 9]	0.050
Interference 6.54 (2.57, 1.54) 2.27 (5.32, 1.56) 0.54 5 Memory central CD4* T cells 45.6 (17.0) 47.5 (13.1) 0.730 Memory effector CD4* T cells 23.0 (12.4) 22.1 (13.0) 0.846 Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Effector CD/ ⁺ T cells	0.88[0.20.3.34]	2 27 [0 52:5 66]	0.401
Memory effector CD4* T cells 23.0 (12.4) 22.1 (13.0) 0.846 Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.339 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Memory central $CD4^+$ T cells	45.6 (17.0)	475(131)	0.545
Th1 cells 18.4 [13.5;24.2] 19.8 [15.9;31.5] 0.468 B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] CD14 ⁺ monocytes 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Memory effector CD4 ⁺ T cells	23.0 (12.4)	22.1 (13.0)	0.846
B cells 10.0 (5.20) 6.67 (5.48) 0.107 Innate immune cells, mean (±SD) or median [IQR] CD14 ⁺ monocytes 7.34 (2.06) 6.60 (1.17) 0.196 Eosinophils 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Th1 cells	18.4 [13.5:24.2]	19.8 [15.9:31.5]	0.468
Innate immune cells, mean (±SD) or median [IQR] 7.34 (2.06) 6.60 (1.17) 0.196 CD14* monocytes 0.89 [0.27;1.33] 0.73 [0.61;0.80] 1.000 Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.339 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	B cells	10.0 (5.20)	6.67 (5.48)	0.107
CD14* monocytes7.34 (2.06)6.60 (1.17)0.196Eosinophils0.89 [0.27;1.33]0.73 [0.61;0.80]1.000Neutrophils61.8 (9.13)61.9 (11.6)0.979NK cells10.0 [5.72;13.4]7.70 [5.29;17.2]0.939NKT-like cells1.24 [0.98;1.96]2.34 [1.29;4.02]<0.5	Innate immune cells, mean (\pm SD) or median [IQR]			
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Neutrophils 61.8 (9.13) 61.9 (11.6) 0.979 NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] <0.05	Eosinophils	0.89 [0.27;1.33]	0.73 [0.61;0.80]	1.000
NK cells 10.0 [5.72;13.4] 7.70 [5.29;17.2] 0.939 NKT-like cells 1.24 [0.98;1.96] 2.34 [1.29;4.02] < 0.05	Neutrophils	61.8 (9.13)	61.9 (11.6)	0.979
INKI-IIKe cells I.24 [0.98;1.96] 2.34 [1.29;4.02] < 0.05	NK cells	10.0 [5.72;13.4]	7.70 [5.29;17.2]	0.939
	INK I -IIKE CEIIS	1.24 [0.98;1.96]	2.34 [1.29;4.02]	< 0.05

PPF: progressive pulmonary fibrosis; IQR: interquartile range; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; DL_{CO} : diffusing capacity for carbon monoxide; ILD: interstitial lung disease; AID-ILD: autoimmune disease-associated interstitial lung disease; HP: hypersensitivity pneumonitis; IIP: idiopathic interstitial pneumonia. Differences in the distribution between groups were assessed using "compareGroups" R package. A Shapiro test was performed for each variable and the appropriate statistic test was selected accordingly to their distribution. Data are expressed as mean ± standard deviation (SD), median [and IQR], or *n* (and %). The significance of bold p value is < 0.05.

in PPF patients there was a negative correlation between FVC and CD8 T cells (Fig. 1D), which further supports that an accelerated aging of immune response is present in PPF and associates to lung function impairment. This is novel information since, to date, a link establishing interactions between immune cells and functional impairment in PPF had not been established.

Among potential limitations, we acknowledge the limited size of our cohorts. Consequently, undertaken larger studies become crucial to validate these findings. Moreover, it would be valuable to explore the correlation of these findings with the immunological profile in bronchoalveolar lavage or lung biopsies in further studies.

In summary, this study shows that in patients with f-ILDs several immune populations in circulating blood, skewed toward an aged and exhausted immune profile, relate to lung function impairment at diagnosis, and that PPF is associated with an increased cytotoxic immune response. Collectively, these findings highlight biomarkers of potential clinical utility, although we acknowledge that these observations will have to be confirmed in larger cohorts.



Fig. 1. (A) Significant correlations between both, forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DL_{co}), and the different subtypes of T cell compartment in patients with fibrosing interstitial lung diseases (ILDs); FVC was negatively correlated with $CD8^+HLA-DR^+$ T cells (r = -0.53; p = 0.001) and central memory CD4 T cells (r = -0.39; p = 0.024); FVC and DL_{co} were negatively related with the percentage of T helper type 1 (Th1) cells (r = -0.59; p = 0.0002; and r = -0.43; p = 0.014, respectively) and CD4⁺CD28⁻HLA-DR⁺ (r = -0.43; p = 0.016; and r = -0.041; p = 0.021, respectively). (B) Comparison of % NKT-like cells in non-PPF (blue box) and PPF patients (pink box). (C) Flow cytometry gating scheme to identify NKT-like cells. (D) Correlation between FVC and CD8 T cells in patients with progressive pulmonary fibrosis (PPF) (n = 11) vs non-PPF (n = 22).

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Conflict of Interest

FH-G and JS reports honoraria for lectures, educational events, and support for attending meetings from Roche and Boehringer-Ingelheim, outside the submitted work. XA-R reports honoraria for educational events and support for attending meetings from Boehringer-Ingelheim, outside the submitted work. JS discloses honoraria for lectures, educational events, and support for attending meetings from Astra Zeneca, Gebro, and GSK; consulting fees from Boehringer-Ingelheim and Alofarma, and grants from Roche and Boehringer-Ingelheim, all outside the submitted work. RF reports honoraria for lectures and support for attending meetings from Chiesi and Zambon; grants from GSK, Astra Zeneca, Menarini; consulting fees from GSK, all outside the submitted work. The remaining authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2023.06.009.

References

- 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;205:e18–47, http://dx.doi.org/10.1164/rccm.202202-0399st.
- Molina-Molina M, Buendia-Roldan I, Castillo D, Caro F, Valenzuela C, Selman M. Diagnostic and therapeutic developments in progressive pulmonary fibrosis. Arch Bronconeumol. 2022;58:418–24, http://dx.doi.org/10.1016/j.arbres.2021.12.006.
- Roca J, Sanchis J, Agusti-Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, et al. Spirometric reference values from a Mediterranean population. Bull Eur Physiopathol Respir. 1986;22:217–24.
- Lukas Yani S, Keller M, Melzer FL, Weinberger B, Pangrazzi L, Sopper S, et al. CD8(+)HLADR(+) regulatory T cells change with aging: they increase in number, but lose checkpoint inhibitory molecules and suppressive function. Front Immunol. 2018;9:1201, http://dx.doi.org/10.3389/fimmu.2018. 01201.
- Cossarizza A, Ortolani C, Paganelli R, Barbieri D, Monti D, Sansoni P, et al. CD45 isoforms expression on CD4+ and CD8+ T cells throughout life, from newborns to centenarians: implications for T cell memory. Mech Ageing Dev. 1996;86:173–95, http://dx.doi.org/10.1016/0047-6374(95) 01691-0.
- Gilani SR, Vuga LJ, Lindell KO, Gibson KF, Xue J, Kaminski N, et al. CD28 down-regulation on circulating CD4 T-cells is associated with poor prognoses of patients with idiopathic pulmonary fibrosis. PLoS One. 2010;5:e8959, http://dx.doi.org/10.1371/journal.pone.0008959.
- Spagnolo P, Tonelli R, Samarelli AV, Castelli G, Cocconcelli E, Petrarulo S, et al. The role of immune response in the pathogenesis of idiopathic pulmonary fibrosis: far beyond the Th1/Th2 imbalance. Expert Opin Ther Targets. 2022;26:617–31, http://dx.doi.org/10.1080/14728222.2022.2114897.
- Bergantini L, Cameli P, d'Alessandro M, Vagaggini C, Refini RM, Landi C, et al. NK and NKT-like cells in granulomatous and fibrotic lung diseases. Clin Exp Med. 2019;19:487–94, http://dx.doi.org/10.1007/s10238-019-00578-3.
- Pereira BI, Devine OP, Vukmanovic-Stejic M, Chambers ES, Subramanian P, Patel N, et al. Senescent cells evade immune clearance via HLA-Emediated NK and CD8(+) T cell inhibition. Nat Commun. 2019;10:2387, http://dx.doi.org/10.1038/s41467-019-10335-5.
- Faner R, Rojas M, Macnee W, Agusti A. Abnormal lung aging in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:306–13, http://dx.doi.org/10.1164/rccm.201202-0282PP.

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