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**Identification and characterisation of chronic lung allograft dysfunction patients with mixed phenotype:
A single center study**

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Identification and characterisation of chronic lung allograft dysfunction patients with mixed phenotype:

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ABSTRACT

Rationale: Patients can change chronic lung allograft dysfunction (CLAD) phenotype, especially from BOS to mixed phenotype. Our aim was to further characterize these patients.

Method: Mixed CLAD was defined as a restrictive physiology with persistent CT opacities, after initial bronchiolitis obliterans syndrome (BOS) diagnosis. The incidence, prognosis, pulmonary function, radiology, pathology and airway inflammation were compared between patients with restrictive allograft syndrome (RAS) and mixed CLAD.

Result: 268 (44%) patients developed CLAD of which 47 (18%) were diagnosed with RAS 'ab initio', 215 (80%) with BOS and 6 (2%) an undefined phenotype. Twenty-five patients developed a mixed CLAD phenotype (24 BOS to mixed and 1 RAS to mixed). Survival after mixed phenotype diagnosis, was comparable ($p=0.39$) to RAS. More emphysema patients developed a mixed phenotype ($p=0.020$) compared to RAS ab initio, while mixed CLAD patients had a lower FEV₁ ($p<0.0001$) and FEV₁/FVC ($p=0.0002$) at diagnosis compared to RAS ab initio. CT scans in patients with the mixed phenotype demonstrated apical predominance of the opacities ($p=0.0034$) with pleuro-parenchymal fibro-elastosis on histopathology.

Conclusion: We further characterized patients with a mixed phenotype of CLAD. Although the survival after diagnosis was comparable to RAS ab initio patients, there was a difference in demography, pulmonary function, radiology and pathology.

Keywords: Lung transplantation, chronic lung allograft dysfunction, restrictive allograft syndrome, bronchiolitis obliterans syndrome, phenotypes, mixed CLAD

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1. INTRODUCTION

Chronic rejection remains one of the major hurdles limiting long-term survival after lung transplantation¹. Historically, it was assumed that chronic rejection was homogeneous and diagnosis was primarily based on the evolution of the forced expiratory volume in 1 second (FEV₁)². Nowadays, however, it is accepted that multiple phenotypes of chronic rejection exist and chronic lung allograft dysfunction (CLAD) has been defined as an overarching term to capture these multiple manifestations³. This division became most apparent after Sato et al. defined restrictive allograft syndrome (RAS), diagnosed by a decline in total lung capacity (TLC) ≥10% compared to baseline, concomitant with a persistent FEV₁ decline ≥20%, present in approximately 30% of CLAD patients⁴. This syndrome was initially characterized by signs of apical dominant interstitial lung disease on chest CT and pleuro-parenchymal fibro-elastosis, non-specific interstitial pneumonia (NSIP) and organising pneumonia on pathology⁵⁻⁶. More importantly, median survival after diagnosis is significantly worse in RAS compared to bronchiolitis obliterans syndrome (BOS) (1-1.5 vs 3-5 years post diagnosis)^{4,7}. A recent ISHLT consensus document redefined RAS as a persistent decline in FEV₁ ≥20% with restriction (TLC decline ≥10%) AND persistent opacities on imaging (preferably high resolution CT scan)⁸⁻⁹.

Already in their landmark paper, Sato et al. made clear that patients could evolve from one phenotype to another (n=9/156 CLAD patients, 6%). Indeed, 8 patients presented with an initial BOS and evolved to RAS, while one patient did the opposite⁴. In the recently published CLAD guidelines, the combination of obstruction, restriction, and persistent lung opacities is usually defined as the mixed phenotype of CLAD⁸. In a two-center study, we identified 28 patients (53% of all RAS patients), who first presented with BOS and later evolved to RAS (now called mixed phenotype), although this did not have any prognostic impact compared to patients developing RAS *ab initio*¹⁰. Most other studies assessing and defining restrictive forms of CLAD focused on the initial presentation (spirometry, body plethysmograph, imaging) at diagnosis, but did not take subsequent changes to a mixed phenotype in the follow up into account¹¹⁻¹⁴. Given the lack of characterization of these patients with the mixed CLAD phenotype, we sought to investigate the incidence, prognosis, pulmonary function evolution, radiology, pathology and airway inflammation in these mixed phenotype patients by a thorough comparison with patients who were diagnosed with the RAS phenotype when they first developed CLAD.

2. MATERIAL & METHODS

2.1 Patient inclusion

We retrospectively assessed chest CT-scans of all patients transplanted between 2001 and 2015. Follow-up was censored on September 1st, 2018. We only included patients undergoing a first double lung or heart-lung transplant, with a post-transplant survival > 6 months and with a definite CLAD diagnosis. CLAD was diagnosed in the presence of a persistent decline in $FEV_1 \geq 20\%$ compared to the mean of the two best post-transplant values in the absence of other identifiable causes for graft failure, according to recent guidelines (8). In the majority of the cases, the diagnosis of RAS was made based on an additional decline in TLC $\geq 10\%$ compared to baseline, or when TLC was unavailable, using a decline in forced vital capacity (FVC) $\geq 20\%$ compared to baseline, in both situations with concomitant persistent or evolving (≥ 3 month) pleuro-parenchymal opacities on chest CT scan, for which no other cause could be identified (9). Patients were also classified as RAS or mixed when survival post diagnosis was <3 months, only if pulmonary function demonstrated restriction and explant lungs showed pathology compatible with interstitial/pleural fibrosis (RAS, n=2; mixed, n=1). Patients, who after initial CLAD diagnosis with a BOS phenotype, developed persistent opacities (i.e. ≥ 3 months, not responding to therapy, without another identifiable cause) *and* a TLC decline $\geq 10\%$ compared to the last available BOS TLC value *and/or* an additional decline in FVC $\geq 20\%$ were defined as patients with a mixed phenotype⁸. Pulmonary function in these patients was evaluated at 4 fixed time points: best post LTx, at BOS diagnosis, last pulmonary function before diagnosis of mixed phenotype (last BOS) and at diagnosis of mixed phenotype. Our routine post-transplant follow-up includes a 3-4 monthly visits to the outpatient clinic with spirometry, with a yearly CT scan and pulmonary function testing using bodyplethysmography to measure TLC and residual volume (RV), and in between also whenever deemed necessary for clinical indications. Anti-HLA antibodies were detected by Luminex technology according to the institution's protocol and are considered negative if MFI <500 and positive if MFI ≥ 500 . A positive screening was followed by the identification of the HLA antibody to define it as donor specific antibodies (DSA) or not.

2.2 Radiology, pathology and bronchoscopy

Chest CT scan at diagnosis of RAS or mixed phenotype was reviewed for the predominant location of parenchymal opacities (apical vs basal vs diffuse) and for the presence/increase of airtrapping compared to the previous CT by a board-certified radiologist. Similarly, CT scans were used to determine how long opacities were already present before diagnosis of CLAD (RAS phenotype)⁸⁻⁹. Broncho-alveolar lavage

(BAL) with accompanying transbronchial biopsies (TBBs) was performed within 2 months of diagnosis of CLAD (BOS, RAS and mixed phenotype) using 2x 50 ml of saline, as described elsewhere¹⁰. The returned BAL fractions were pooled and processed for total and differential cell counting and routine clinical microbial screening. TBBs were assessed for acute rejection (Grade A) or lymphocytic bronchiolitis (Grade B), according to ISHLT guidelines¹⁵. Lung biopsies extracted during redo-transplantation, autopsy or video assisted thoracic surgery were reviewed by an experienced lung transplant pathologist (JHVdT). A proportion of these lungs have been used for a previous study⁶.

2.3 Statistics

Results are displayed as median (IQR) or n (%). Graft survival is a composite endpoint consisting of either death or redo transplantation. Survival was compared using Kaplan-Meier analysis. Comparison of continuous data between RAS and BOS evolving to RAS were performed using Mann Whitney testing. Discrete data were compared using contingency tables. Graph Pad Prism 7.0 was used for all analyses. A $p < 0.05$ was considered significant.

3. RESULTS

3.1 Patient groups

Patient selection is shown in figure 1. Forty-four % of our cohort developed CLAD of which 47 (18%) were diagnosed with RAS (TLC decline $\geq 10\%$, $n=29$; FVC decline ≥ 20 , $n=18$) and 215 (80%) were initially diagnosed with BOS. Six patients (2%) presented with obstructive physiology and persistent radiographic opacities, however, without additional decrease in FVC or TLC (i.e. no restriction), and were hence classified as “undefined phenotype” (8). Twenty-four patients (9% of entire CLAD cohort; 11 % of the entire BOS cohort) evolved from an initial BOS phenotype to a mixed phenotype (representative example in figure 2). One patient evolved from RAS to a mixed phenotype, with partial resolution of the opacities and a progressively more obstructive spirometry with increasing TLC. Given the limited power of these undefined and RAS to mixed phenotype, we will further focus on the comparison between the “classical” mixed phenotype (initial BOS phenotype, evolving to mixed, $n=24$) versus RAS “ab initio” ($n=46$). Table 1 gives an overview of these different CLAD phenotypes and their characteristics.

Patient characteristics are shown in table 2. While there is no difference in type of transplant, gender and age, there were significantly more patients with underlying COPD/emphysema diagnosis and a lower number of patients with interstitial lung disease ($p=0.038$) and cystic fibrosis ($p=0.012$) that evolved from

the BOS to the mixed phenotype ($p=0.020$). In 8 out of 22 (36%) patients in the mixed phenotype group and in 14 out of 40 RAS patients (35%), circulating DSA were detected ($p=1.0$), mostly directed against HLA DQ. In none of the cases there was a definite AMR diagnosis given the absence of C4d staining or presence of typical histology. In the remaining patients no assessment of HLA antibodies was available.

Median time to diagnosis of mixed phenotype was longer in patients with initial BOS diagnosis (4.7 Y, IQR 3.8-6.6 Y) compared to the RAS 'ab initio' group (3.3 Y, IQR 1.5-6.9 Y) ($p=0.036$). Median survival after CLAD diagnosis was longer in the mixed compared to the RAS phenotype patients (4.1 Y vs 1.2 Y, $p=0.0084$) (figure 3A). This is explained by the lag time between initial BOS and mixed phenotype diagnosis which was 1.9 Y (IQR 0.9-4.3Y). After diagnosis of the mixed phenotype, survival was similar in both groups (0.8 Y in mixed versus 1.2 Y in the RAS group, $p=0.37$, figure 3B).

In 10 (42%) of the mixed phenotype patients, no clear trigger was identified, while 13 patients had evidence of infection prior to the mixed phenotype diagnosis and one patient had an episode of antibody-mediated rejection between CLAD and mixed diagnosis. Those patients with an identifiable trigger tended to experience worse prognosis following diagnosis compared to the ones without identifiable trigger ($p=0.052$) (figure 3C).

3.2 Pulmonary function

Pulmonary function evolution of patients with the mixed phenotype is shown in figure 4. Seven patients did not change their CLAD stage from the initial BOS diagnosis to the moment they became mixed. The other 17 patients experienced a further increase in CLAD stage from initial BOS to mixed diagnosis ($n=5$ from CLAD1 to 2; $n=5$ from CLAD1 to 3; $n=3$ from CLAD 1 to 4; $n=2$ from CLAD 2 to 3 and $n=2$ from CLAD 2 to 4)(8).

All patients show a loss in $FVC/FVC_{best} >20\%$ and $TLC/TLC_{lastBOS} >10\%$ compared to baseline at transition from BOS to mixed. However, comparing FVC and TLC at the last available BOS time to the measurement at diagnosis of mixed, showed that only 9 out of 24 patients (38%) had an additional FVC decline $>20\%$, while 19/21 (90%) patients showed a decrease in $TLC >10\%$ (in 3 patients not enough TLC measurements available).

Comparing the mixed patients to the patients who developed RAS ab initio, there was no difference in the absolute FVC ($p=0.51$) and TLC ($p=0.69$) at diagnosis, but there was a significantly lower FEV_1 ($p=0.0017$) and FEV_1/FVC ($p=0.0002$) in the mixed phenotype, which is explained by the natural progression of BOS before mixed diagnosis (table 3).

Comparing the mixed phenotype to the entire cohort of BOS patients, we found that the FEV₁ and FVC was similar at BOS diagnosis (p=0.24 and p=0.36) and 3 months (p=0.47 and p=0.35) following BOS diagnosis, suggesting a similar trajectory before the eventual phenotype switch to mixed.

3.3 Radiology and pathology

CT at diagnosis showed an apical predominance in the majority of mixed phenotype patients (18/24, 75%), with a low prevalence of basal (n=3, 12.5%), and diffuse (n=3, 12.5%) opacities. In contrast, RAS patients showed no predominance in the location of the opacities, with a similar degree of apical (n=15, 32.5%), basal (n=16, 35%) and diffuse predominance (n=15, 32.5%) (p=0.0034). Before RAS diagnosis, there was no difference in the number of patients with pre-existing CT opacities (i.e. undefined phenotype of CLAD prior to RAS or mixed diagnosis) with 20/46 in the RAS group and 8/24 in mixed group (p=0.41). Pre-existing CT opacities did not affect post-diagnosis survival (p=0.22 for mixed phenotype and p=0.99 for RAS ab initio).

The presence or increase in the amount of air trapping in BOS, mixed and RAS lungs was also not discriminatory between the different phenotypes as a similar proportion of patients showed stable, increased or no air trapping in the different groups (p=0.66).

Pathology at redo-transplantation/autopsy or video assisted thoracic surgical biopsy was available in 10 patients that evolved from BOS to mixed (42%) and 21 RAS ab initio patients (46%). There were no differences in the pathological patterns between both groups. (table 3)

3.4 Broncho-alveolar Lavage (BAL) and biopsy results

BAL at RAS diagnosis was available in 41 RAS patients (89%) and in 18 mixed phenotype patients (72%) around time of diagnosis (± 2 months). There was no statistical significant difference in the total cell count (p=0.20), nor in the relative abundance of macrophages (p=0.068), neutrophils (p=0.46) or lymphocytes (p=0.17). The % eosinophils in BAL was higher in RAS compared to the mixed group (1.4% IQR 0.0-4.8 versus 0.1% IQR 0.0-0.8; p=0.020). Transbronchial biopsies showed evidence of mild acute perivascular rejection (A $\geq$ 2) more frequently in RAS (14/36) compared to the mixed phenotype (1/14) (p=0.028), but no difference in lymphocytic bronchiolitis (7/41 with LB in the RAS group and 3/16 in the mixed, p=1.0).

DISCUSSION

In this study, we compared the incidence, demographics, prognosis, pulmonary function evolution, radiology and pathology in the largest cohort of RAS patients and patients that evolved from BOS to mixed phenotype published so far. This BOS to mixed phenotype evolution was more frequently observed in patients transplanted for emphysema. Additionally, the mixed phenotype (BOS->mixed) displayed an apical predominance of interstitial/pleural opacities, with pleuro-parenchymal fibro-elastosis on pathology.

Survival was similar in mixed phenotype patients and RAS 'ab initio' patients following RAS/mixed diagnosis, although, there was a tendency for a difference in the death/ redo transplant rate between both groups. This is likely due to the higher age at mixed diagnosis making these not eligible for a redo transplant per our center protocol regarding redo transplantations.

Since most studies performed so far have investigated the prognostic value of different parameters of pulmonary function tests at diagnosis of CLAD (obstructive, restrictive) and did not take further evolution over time into account, patients that change their CLAD phenotype remain ill-defined. The finding that 11% of patients with a BOS diagnosis change to the mixed phenotype, indicates that it is important to take evolution after CLAD onset into account; otherwise only one patient changed from RAS to mixed.

Based on our observations, a decline in TLC may be the most appropriate pulmonary function test to diagnose the mixed phenotype in the presence of prior BOS. Since FVC usually already decreases during subsequent BOS progression, we found an additional decline in $FVC > 20\%$ only in a minority of the patients. In contrast, TLC mostly remains stable or even increases following BOS diagnosis¹⁶, therefore a TLC decline $\geq 10\%$ is evident in almost all patients when they evolve from BOS to the mixed phenotype. However, not the best postoperative TLC should be used to calculate this decline, but rather the last TLC available during BOS progression, as suggested by the new guidelines⁸, since TLC usually increases in BOS patients¹⁶. Therefore, this favours performing routine plethysmography in the follow-up of lung transplant patients, also after CLAD (BOS) has been diagnosed. This discrepancy between FVC and TLC decline to diagnose a phenotype evolution is likely to be explained by the increase in air trapping related to BOS progression, which can also lead to a decreased FVC in the absence of a TLC decline.

We also provide confirmation that 1 patient in our cohort evolved from an initial RAS phenotype⁹ to the mixed phenotype, where CT opacities partly resolved and pulmonary function became more obstructive with a concomitant TLC increase. Although our experience is limited, it is striking that our patient underwent plasmapheresis and is still alive 2 years after initial decrease in TLC and appearance of

opacities, suggesting that this might have been a sort of antibody mediated rejection without circulating DSA nor positive C4d staining on transbronchial biopsies. In retrospect, therefore, it seems strange that this patient suffered from RAS ab initio, as resolution of fibrosis is not expected, however, at the moment of RAS diagnosis all criteria, according to the recent guidelines were fulfilled (i.e. persistent decrease in FEV₁, FVC and TLC and persistent opacities for 6 months, despite different treatment options)⁹. Surprisingly, we also found a small number of CLAD patients (n=6, with declining FEV₁>20% and obstructive spirometry) *and* persistent CT opacities, without an additional decrease in FVC and TLC. This is not compatible with BOS nor RAS but is now accepted as an undefined phenotype⁸. It is not unlikely that these patients will develop a progressive decrease of FVC or TLC during further follow-up and are prone to develop a mixed phenotype, although this will need further evaluation. We also found that a large proportion of both the RAS ab initio patients (20/46) and mixed patients (8/24) had pre-existing CT opacities (but did not qualify for RAS/mixed at that time because of absence of the pulmonary function criteria)¹⁷.

We found important differences in the location of CT opacities and pathology at explant between RAS and mixed phenotype patients. The mixed group demonstrated a predominant apical-dominant fibrosis with the presence of pleuro-parenchymal fibro-elastosis (PPFE), which was initially considered to be the typical pathological surrogate of RAS⁵. However, the present study demonstrated that in patients developing pure RAS, only 40% showed PPFE, in contrast to 94% in the study by Ofek et al. The apical predominance in the mixed phenotype patients is difficult to explain, although it is similar to the apical predominance in idiopathic PPFE, where it is speculated that this pattern can be explained by hypoperfusion and ischemia of the apical areas¹⁹. Obliterative bronchiolitis (OB) was found in almost all specimens with explant material available, consistent with previous observations^{5-6,20}. The fact that in some specimens no OB lesions were found, may be attributed to sampling bias.

Current data in our study does not provide compelling evidence that the pathophysiology is different in RAS and patients that evolve from BOS to a mixed phenotype. However, this was not the primary aim of this study and further studies are needed to investigate the pathophysiological mechanisms of the different CLAD phenotypes. Within this study, we observed that the BAL at diagnosis was similar except for increased eosinophilia in the RAS group, while a similar (relatively high) presence of DSA in blood was seen. Indeed 32-35% of patients had circulating DSA, which is higher compared to our entire cohort (19%)²¹. This indicates that humoral immunity might be involved in the development of RAS-like fibrosis²². Interestingly, the number of high-grade acute rejections was somewhat higher in the mixed group, indicating this could be a potential trigger for mixed development.

Given that a lot of risk factors (e.g. acute rejection, colonisation, DSA^{21,23}) are shared between BOS and RAS phenotype, combined with this report that patients change phenotype, renders support to the claim that BOS and RAS may be a continuum of the same disease. In contrast, the trigger identified in the majority of mixed phenotype patients suggests that RAS development may be independent of BOS. However, larger multi centric studies are needed to clarify this issue.

Limitations of the present study include its monocentric approach, the limited number of patients included for some of the comparisons and the absence of specific parameters such as pathology in some patients. However, this study reports on the largest cohort of patients with a RAS/mixed phenotype of CLAD published thus far, not only including measurements at diagnosis, but also serial follow-up.

In conclusion, we compared patients with mixed CLAD to RAS ab initio patients. This mixed phenotype occurs mostly in emphysema patients, who first develop BOS and then evolve to a mixed phenotype defined by an additional decrease in TLC, apical predominance of CT opacities with PPFE on pathologic examination, the reverse evolution (RAS to mixed) is very infrequent. Survival from the moment the mixed phenotype was diagnosed was similar to RAS 'ab initio' patients. The survival was better compared to RAS ab initio patients if the survival is plotted from diagnosis of CLAD (BOS) on.

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Figure legends

Figure 1: Flowchart indicating patient inclusion criteria. From 268 patients with definite CLAD diagnosis, 47 showed an initial RAS diagnosis, 215 an initial BOS diagnosis and 6 showed an undefined CLAD phenotype. 25 patients eventually showed a mixed phenotype with 24 patients changing from BOS to mixed and 1 patients from RAS to mixed. LTx: lung transplantation; SSLTx: sequential single lung transplantation; CLAD: chronic lung allograft dysfunction; TLC: total lung capacity; FVC: forced vital capacity.

Figure 2: A: Pulmonary function evolution in a CLAD patient who evolved from BOS to mixed, where the initial FVC remained stable but which deteriorated later during the disease course and a mixed phenotype diagnosis was made. B-E Chest CT scan evolution of the same patient that evolved from BOS to mixed where at first there was no evidence of persistent lung opacities until FVC dropped and severe (sub-)pleural thickening was observed. FEV₁: forced expiratory volume in 1 second; FVC: forced vital capacity; BOS: bronchiolitis obliterans syndrome; CLAD: chronic lung allograft dysfunction; POD: post-operative date.

Figure 3: Survival analysis. A. Survival in patients with the mixed phenotype is longer following CLAD diagnosis compared to the RAS ab initio group. B. The survival in patients from diagnosis of mixed phenotype is comparable to the RAS group. C. Within patients with the mixed phenotype, the ones with an identifiable trigger leading to a change in phenotype tended to have a worse survival compared to the ones without identifiable trigger ($p=0.052$).

Figure 4: Evolution of pulmonary function parameters in patients with the mixed phenotype at different time points before the diagnosis was established. A: Evolution of FEV₁. B: Evolution of the FVC. C: Evolution of FEV₁/FVC. D: Evolution of TLC. FEV₁: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity. The red line indicates the mean values.

Obstructive PFT	restrictive PFT	opacities present	phenotype
no	yes	yes	RAS (n=46)
yes	yes	yes	mixed (n=25)*
yes	no	yes	undefined (n=6)

Table 1: overview of the different diagnostic criteria and CLAD phenotype. PFT=pulmonary function test. * 24 patients evolved from BOS to mixed, 1 patients evolved from RAS to mixed.

Characteristic	Mixed	RAS	
n	24	46	
Type, N (%)			0.41
SSLTx	21 (88%)	43 (93%)	
HLTx	3 (12%)	3 (7%)	
Native lung disease, N(%)			
Emphysema	15 (63%)	14 (30%)	0.020
ILD	2 (8%)	15 (33%)	0.038
Sarcoidosis	2 (8%)	2 (4%)	0.60
CF+non-CF Brect	0 (0%)	10 (22%)	0.012
PAT	3 (14%)	4 (9%)	0.68
Others	2 (8%)	1 (2%)	0.27
Male gender, N (%)	12 (50%)	27 (59%)	0.61
Donor age, Y	47 (32-55)	46 (25-57)	0.94
Age at transplant, Y	54 (41-58)	49 (25-57)	0.23
Age at diagnosis, Y	59 (49-64)	52 (32-62)	0.076

Table 2: patient characteristics. SSLTx: double lung transplantation; HLTx: heart lung transplantation; ILD: interstitial lung disease; CF: cystic fibrosis; Brect: bronchiectasis; PAT: pulmonary arterial hypertension.

Characteristic	Mixed	RAS	
n	24	46	
Outcome			0.06
Alive	3 (12%)	11 (24%)	
Dead	19 (76%)	21 (45%)	
Redo transplantation	3 (12%)	13 (28%)	
Pulmonary function best			
FEV1 (L)	2.9 (2.2-3.6)	3.1 (2.4-3.6)	0.72
FEV1 (%pred)	101 (87-111)	95 (75-111)	0.49
FVC (L)	3.8 (2.9-4.9)	3.7 (3.2-4.5)	0.82
FEV1/FVC	0.76 (0.74-0.86)	0.78 (0.73-0.87)	0.99
Pulmonary function at diagnosis			
FEV ₁ (L)	1.27 (0.96-1.74)	1.84 (1.37-2.40)	0.0017
FEV ₁ /FEV _{1best} (%)	51 (37-62)	68 (60-73)	<0.0001
FVC (L)	2.33 (1.73-3.18)	2.65 (1.83-3.30)	0.51
FVC/FVC _{best}	65 (53-75)	69 (64-78)	0.14
FEV1/FVC	0.60 (0.48-0.68)	0.78 (0.68-0.84)	0.0002
TLC	3.96 (3.53-5.16)	4.08 (3.2-4.8)	0.69
TLC/TLC _{best}	0.81(0.78-0.84)	0.83 (0.73-0.88)	0.57
Time in BOS before mixed (Y)	1.9 (0.9-4.3)	Not applicable	
Localisation of CT opacities			0.034
Apical	19 (76%)	15 (32.5%)	
Basal	3 (12%)	16 (35%)	
Diffuse	3 (12%)	15 (32.5%)	
Pathological pattern at explant			
Available specimens	10 (40%)	21 (46%)	
AFOP	3 (30%)	7 (33%)	0.85
NSIP	1 (10%)	6 (29%)	0.25
Emphysema	2 (20%)	4 (19%)	0.95
OP	2 (20%)	11 (52%)	0.088
PPFE	7 (70%)	8 (38%)	0.097
OB	8 (80%)	18 (86%)	0.69
Acute rejection			0.16
A0	1 (10%)	6 (29%)	

A1	1 (10%)	7 (33%)	
A≥2	6 (60%)	5 (24%)	
A not gradable	2 (20%)	3 (14%)	
BAL at diagnosis			
Available specimens	18 (72%)	41 (89%)	
Total cell count	0.073 (0.044-0.23)	0.13 (0.077-0.38)	0.20
Neutrophils (%)	13.9 (4.6-30.0)	18.5(6.2-53.2)	0.46
Macrophages (%)	80.3 (60.4-85.6)	57.0 (24.3-82.4)	0.068
Lymphocytes (%)	4.3 (2.0-10.7)	7.0 (2.5-18.5)	0.17
Eosinophils (%)	0.1 (0-0.8)	1.4 (0-4.8)	0.020

Abbreviations: FEV1: forced expiratory volume in second; FVC: forced vital capacity; TLC: total lung capacity; AFOP: acute fibrinous organizing pneumonia; NSIP: non-specific interstitial pneumonia; OP: organizing pneumonia; PPFE: pleuro-parenchymal fibro-elastosis; OB: obliterative bronchiolitis; BAL: broncho-alveolar lavage. The pathological patterns are not mutually exclusive.

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