ONLINE SUPPLEMENT

Late-onset 'acute fibrinous and organizing pneumonia' impairs long-term lung allograft function and survival

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Extended Methods

Transplant monitoring schedule

All LTx recipients received routine follow-up visits at fixed time points. The standard follow up protocol is as following: 2/w during the first 4 weeks after discharge, then 1/w until 8 weeks post-LTx, 1/2w until 12 weeks post-LTx, 1/4w until 6 months post-LTx, 1/6w until 12 months post-LTx, and thereafter 1/12w. In addition, patients performed home spirometry and were instructed to come to the outpatient clinic in case of fever or >10% FEV1 decline. Each patient contact included complete history taking and physical examination as well as blood, urine, sputum and pharyngeal swab cultures (if symptomatic), spirometry and chest radiography. In addition, chest CT and bronchoscopic evaluation with broncho-alveolar lavage (BAL) was performed at discharge and at 3, 6, 12, 18 and 24 months after LTx, or whenever clinically indicated. Transbronchial biopsies (at least 5 tissue fragments/procedure) were routinely obtained at discharge and at 3 months post-LTx, or whenever clinically indicated (i.e. unexplained fever, suspicion of infection/rejection, >20% FEV1 drop, radiological abnormalities)

Transbronchial biopsy preparation

All biopsy specimens were prepared according to the routine clinical protocol, formalin fixed, and paraffin embedded. Standard procedure included obtaining 5 µm sections from at least 3 levels of the paraffin block.

Radiologic assessment

Chest CT at AFOP diagnosis was scored for the presence of radiological opacities (i.e. nodular opacifications, GGOs, crazy paving pattern, consolidation), the localization of infiltrates (diffuse, apical, basal) and for the presence of air trapping and pleural effusion.

Histopathologic assessment

Acute rejection and lymphocytic bronchiolitis were graded according to the 2007 grading scheme from the International Society for Heart and Lung Transplantation (1). The presence of concomitant intra-alveolar red blood cells (RBCs), hemosiderin-laden macrophages and histologic evidence of infection was also reviewed.

Extended results

Treatment regimens in AFOP patients

Eight (27%) patients with *late* AFOP were treated with plasmapheresis and intravenous immune globulin therapy treatment, compared to 2 (8%) patients with *early* AFOP (p=0.16). Methylprednisolone (500mg/3d) was administered to 12 patients with *late* AFOP and 1 *early* AFOP patient (p=0.0030); and Rituximab to 5 *late* AFOP patients and no *early* AFOP patients (p=0.059).

Indication biopsies

For a subset of both *early* and *late* AFOP patients, a second biopsy displaying AFOP was present. More precisely, a follow up biopsy with AFOP (>90 days post-LTx) was present in 4 (17%) patients with early AFOP and these patients had a significant lower CLAD-free survival (p=0.0024) compared to early AFOP patients without a later follow up biopsy with AFOP. The median time between the first and the second detection of AFOP was 210 days (IQR: 30-361) for these patients. In addition, 9 of 30 (30%) *late* AFOP patients had a follow up biopsy displaying AFOP, with a median time between the first and second detection of AFOP of 124 days (IQR: 22-141).

Histopathologic findings in early AFOP explant lungs

Explant lung biopsies were available for 2/2 (100%) *early* fibrin/OP patients with graft loss, which displayed multiple pulmonary emboli in one patient and presence of bronchiolitis obliterans lesions, consistent with clinical BOS, in the other patient.

Evidence of infection in BAL fluid

There was concurrent evidence of infection in BAL fluid of 5/24 (21%) *early* AFOP patients (Aspergillus, n=2; Serratia marcescens, n=1; Pseudomonas aeruginosa, n=1; Enterococcus faecium, n=1) and 5/30 (17%) *late* AFOP patients (Aspergillus, n=1; Human Metapneumovirus; n=1; Respiratory Syncytial Virus, n=1; Parainfluenza virus type 1, n=1; Influenza virus type B, n=1).

Supplementary figures

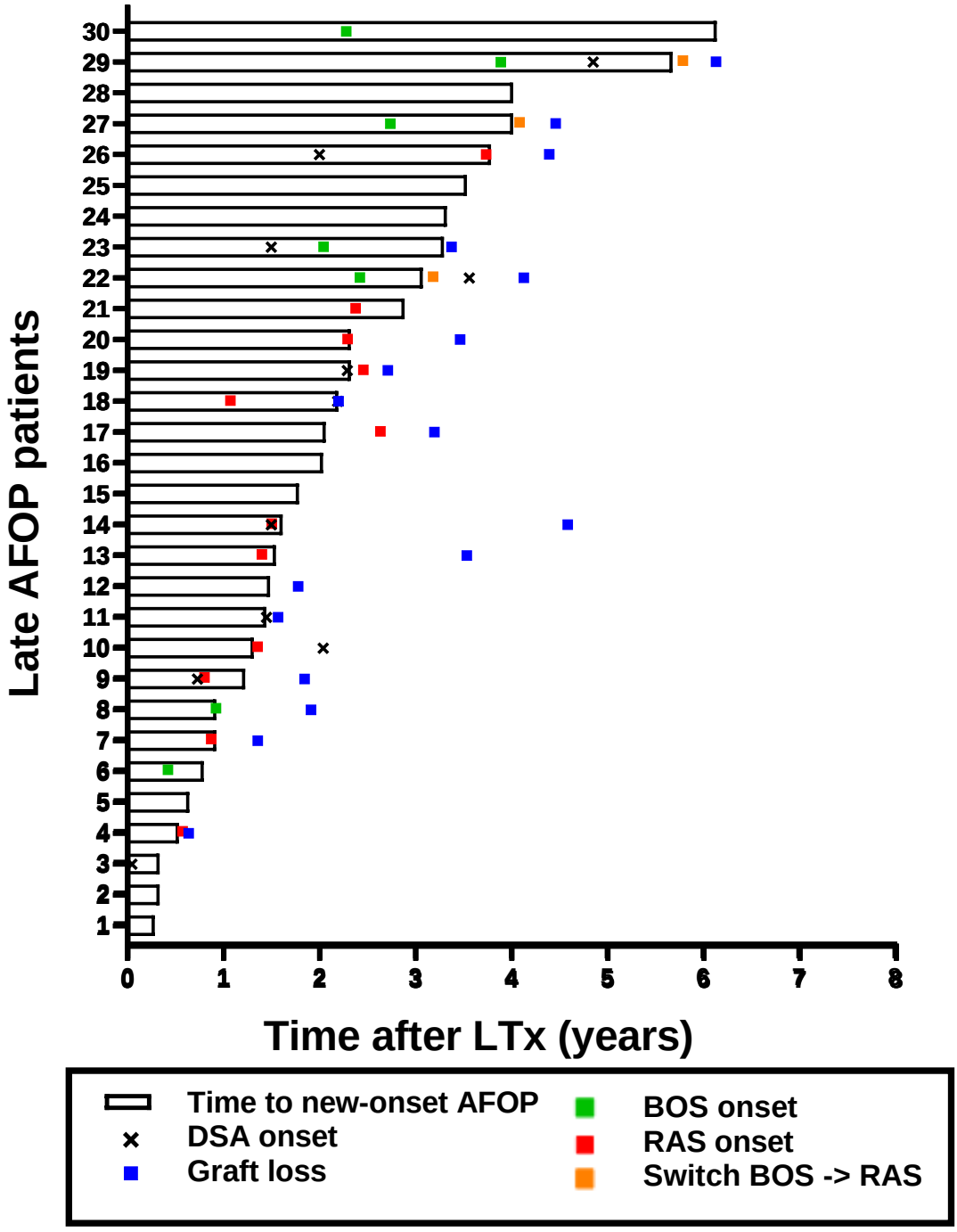

Figure S1. Timeline illustrating the relation between the time to new-onset *late* AFOP, detection of DSAs, CLAD-free and graft survival of individual *late* AFOP patients. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

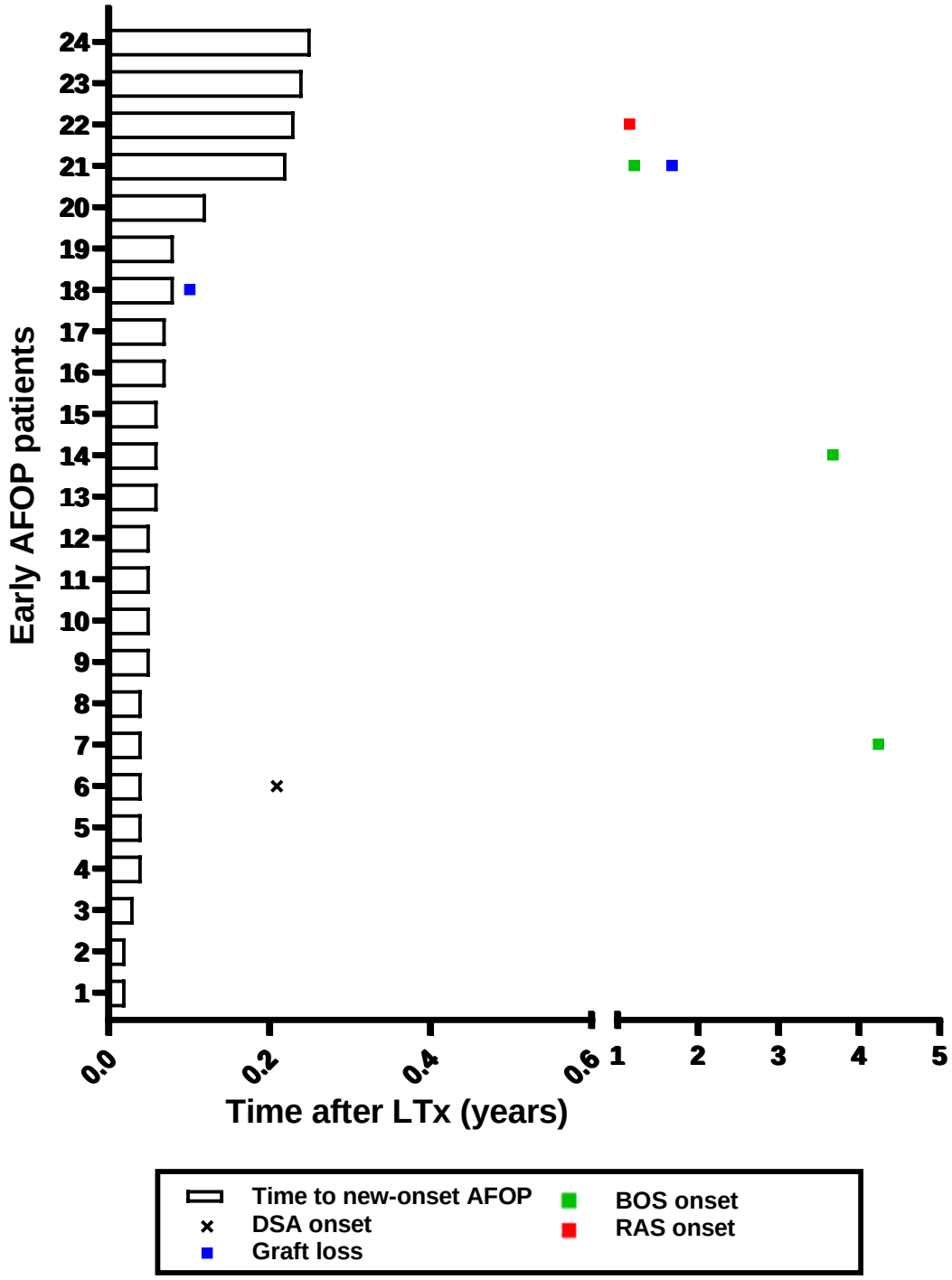

Figure S2. Timeline illustrating the relation between the time to new-onset *early* AFOP, detection of DSAs, CLAD-free and graft survival of individual *early* AFOP patients. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

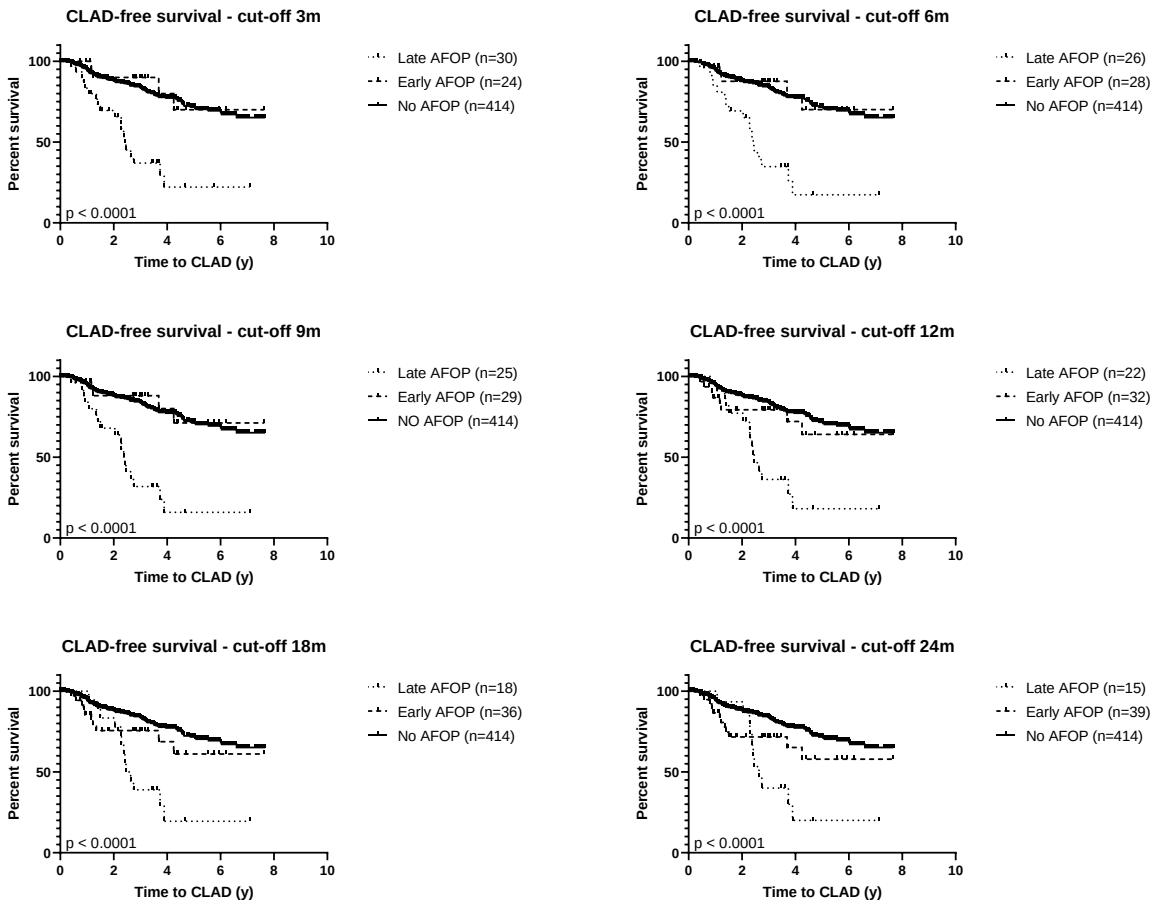

Figure S3. Kaplan-Meier curves illustrating the influence of applying several cut-offs (i.e. 3m, 6m, 9m, 12m, 18m, 24m) on CLAD-free survival. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

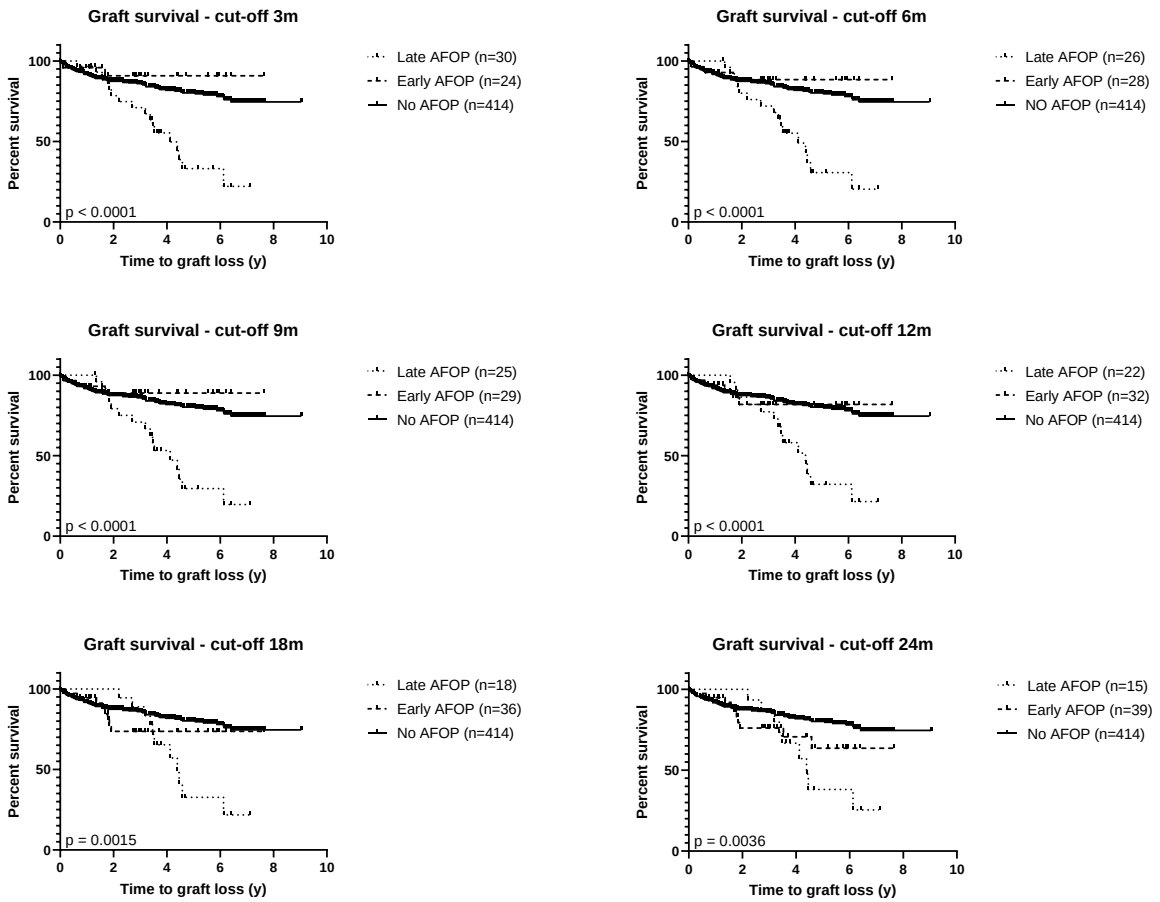

Figure S4. Kaplan-Meier curves illustrating the influence of applying several cut-offs (i.e. 3m, 6m, 9m, 12m, 18m, 24m) on graft survival. AFOP: acute fibrinous and organizing pneumonia; LTx: lung transplantation.

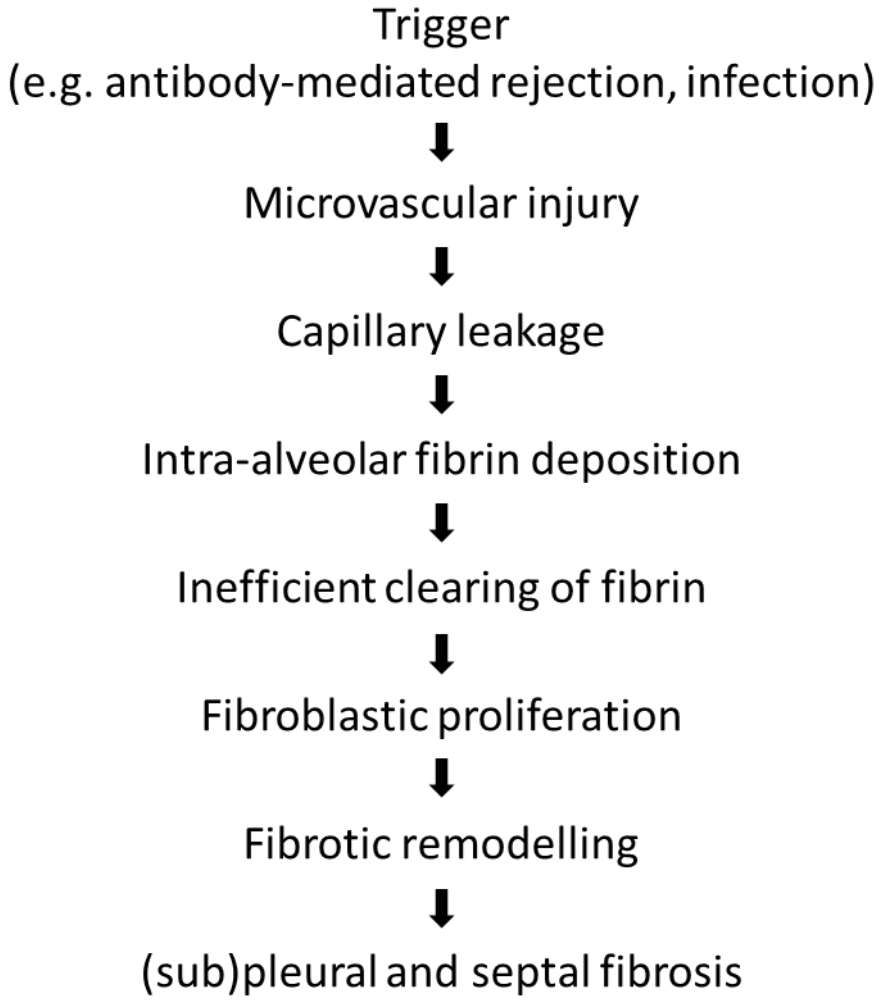

Figure S5. Proposed pathologic cascade leading to the development of restrictive allograft syndrome.

Supplementary tables

Table S1. Laboratory results			
	Early AFOP (n=24)	**Late AFOP (n=30)**	**p value**
BAL, N (%)	19 (79)	23 (77)	
BAL total n cells (x10^6)	4.08 (1.60 – 9.43)	1.69 (0.62 – 8.56)	0.17
Total volume (ml)	42 (35 – 57)	42.50 (36.75 – 53.25)	0.92
Total cells (x10^3/ml)	159 (92 – 272)	69 (36 – 321)	0.10
Macrophages (%)	73.50 (38.50 – 85.60)	54.50 (14.50 – 87.50)	0.24
Lymphocytes (%)	3 (1 – 7)	3.60 (1.60 – 13.80)	0.33
Neutrophils (%)	25.40 (8.50 – 55.00)	28.20 (7.00 – 72.50)	0.69
Eosinophils (%)	0 (0 – 0.40)	0.50 (0 – 5.20)	**0.043**
Peripheral blood			
WBC count (10^9/L)	8.13 (4.73 – 13.77)	7.13 (4.95 – 10.11)	0.55
WBC differentiation, N(%)	18 (75)	29 (97)	
Neutrophils (%)	80.15 (64.50 – 88.05)	74.40 (63.80 – 84.15)	0.29
Neutrophils (10^9/L)	5.20 (2.55 – 7.65)	4.90 (3.30 – 8.25)	0.89
Eosinophils (%)	0.90 (0.18 – 1.78)	1.6 (0.60 – 2.50)	0.054
Eosinophils (10^9/L)	0 (0 – 0.10)	0.10 (0 – 0.20)	**0.045**
Basophils (%)	0.20 (0 – 0.63)	0.20 (0 – 0.40)	0.78
Basophils (10^9/L)	0 (0 – 0)	0 (0 – 0)	0.38
Lymphocytes (%)	10.60 (6.60 – 22.83)	11.90 (6.55 – 23.55)	0.84
Lymphocytes (10^9/L)	0.70 (0.38 – 1.05)	1.10 (0.40 – 1.55)	0.18
Monocytes (%)	6.75 (4.25 – 8.55)	8.8 (7.25 – 10.25)	0.083
Monocytes (10^9/L)	0.40 (0.18 – 0.83)	0.70 (0.40 – 0.80)	0.13
CRP (mg/L)	14.5 (2.93 – 71.63)	40.75 (9.75 – 97.03)	0.099

Table S1. Data are shown as n (%) or median (interquartile range). The two groups were compared using Mann Whitney test. AFOP: acute fibrinous and organizing pneumomia; BAL: broncho-alveolar lavage; WBC: white blood cell; CRP: C-reactive protein.

Table S2. Multivariate analysis				
	CLAD HR (CI)	**P value**	**Graft loss HR (CI)**	**P value**
Native lung disease				
ILD	0.80 (0.47-1.34)	0.39	1.21 (0.71-2.08)	0.48
CF or BRECT	0.52 (0.19-1.37)	0.18	0.87 (0.32-2.40)	0.79
Other	0.57 (0.22-1.45)	0.24	1.61 (0.72-3.60)	0.24
Emphysema	Reference			
Age at LTx	1.00 (0.98-1.03)	0.77	1.01 (0.98-1.03)	0.61
Type of LTx	0.74 (0.17-3.17)	0.68	1.52 (0.56-4.11)	0.41
Epoch (year of LTx)	1.11 (0.98-1.26)	0.10	1.03 (0.90-1.16)	0.70
Sex	0.88 (0.59-1.32)	0.55	1.14 (0.75-1.74)	0.54
Episodes of acute rejection	1.56 (1.21-2.01)	**0.0005**	1.05 (0.74-1.51)	0.78
Episodes of lymphocytic bronchiolitis	1.59 (1.17-2.16)	**0.003**	1.07 (0.70-1.63)	0.76
Episodes of infection	1.59 (1.01-2.49)	**0.043**	1.21 (0.70-2.10)	0.48
Episodes of CMV infection	1.40 (0.60-3.24)	0.43	1.64 (0.70-3.86)	0.26
DSA (ever vs never)	1.57 (0.95-2.60)	0.08	1.24 (0.71-2.15)	0.45
AFOP				
Early	0.92 (0.33-2.54)	0.87	0.51 (0.12-2.08)	0.35
Late	3.05 (1.76-5.27)	**<0.0001**	3.03 (1.71-5.36)	**0.0001**
No	Reference			

Table S2. Multivariate analysis with CLAD and graft loss as primary outcomes. CLAD: chronic lung allograft dysfunction; HR: Hazard ratio; CI: confidence interval; ILD: interstitial lung disease; CF: cystic fibrosis; BRECT: bronchiectasis; CMV: Cytomegalovirus; LTx: lung transplantation; DSA: donor-specific antibodies; AFOP: acute fibrinous and organizing pneumonia.

Table S3. Patient characteristics						
	Total	**No indication biopsy**	**Indication biopsy (≥1) but never abnormality**	**Indication biopsy with abnormality (≥1)**	**Late AFOP**	**p value**
Patients, N (%)	468	195 (41)	135 (29)	108 (23)	30 (6)	
Age at transplant (years)	57 (45-61)	56 (46 -61)	58 (43-61)	57 (48-61)	55 (44-62)	0.94
Male, N (%)	235 (50)	98 (50)	67 (50)	52 (48)	18 (60)	0.72
Underlying disease, N (%)						0.77
Emphysema	250 (53)	99 (51)	72 (53)	62 (57)	17 (57)	
ILD	94 (20)	40 (21)	24 (18)	23 (21)	7 (23)	
CF or BRECT	72 (15)	30 (15)	24 (18)	13 (12)	5 (17)	
Redo transplant	31 (7)	18 (9)	7 (5)	6 (6)	0	
PHT or Eisenmenger	18 (4)	6 (3)	8 (6)	3 (3)	1 (3)	
Other	3 (1)	2 (1)	0 (0)	1 (1)	0	
Type of transplant, N (%)						0.82
SSLTx	451 (96)	187 (96)	129 (96)	104 (96)	29 (97)	
SSLTx + LiTx	8 (2)	3 (2)	3 (2)	2 (2)	0	
HLTx	6 (1)	3 (2)	1 (1)	1 (1)	1 (3)	
SSLTx + KiTx	2 (0.4)	0	2 (1)	0	0	
SLTx	2 (0.4)	1 (1)	0	1 (1)	0	
HLTx + LiTx	1 (0.2)	1 (1)	0	0	0	
Early mortality (<3m)	18 (4)	18 (9)	0	0	0	**<0.0001**

Table S3. Data are shown as n, n (%) or median (interquartile range). Patient characteristics were compared using Chi Square test; age at transplant was compared using Kruskal-Wallis test. Abnormal findings were considered as presence of acute rejection or infection, but absence of AFOP. AFOP: acute fibrinous and organizing pneumonia; ILD: interstitial lung disease; CF: cystic fibrosis; BRECT: bronchiectasis; PHT: pulmonary hypertension; SSLTx: sequential single lung transplantation; LiTx: liver transplantation; HLTx: heart-lung transplantation; KiTx: kidney transplantation; SLTx: single lung transplantation.

Table S4. Indication biopsies (>90 days after transplantation)	
Patients, N	468
Patients without indication biopsy, N	195 (42)
Patients with indication biopsy, N	273 (58)
Patients with N=1 indication biopsy	123 (26)
Patients with N=2 indication biopsies	63 (13)
Patients with N≥3 indication biopsies	87 (19)
Patients with abnormal indication biopsy (n≥1), N	138 (29)
Patients with acute rejection	54 (12)
Patients with lymphocytic bronchiolitis	49 (10)
Patients with infection	45 (10)
Patients with late new-onset AFOP	30 (6)

Table S4. Data are shown as n or n (%). Number of patients with indication biopsies (>90 days after transplantation) and their results. AFOP: acute fibrinous and organizing pneumonia.

References

1. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection. J Hear Lung Transplant. 2007;26:1229–42.