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Late-onset “acute fibrinous and organising pneumonia” impairs long-term lung allograft function and survival

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LATE-ONSET 'ACUTE FIBRINOUS AND ORGANIZING PNEUMONIA' IMPAIRS LONG-TERM LUNG ALLOGRAFT FUNCTION AND SURVIVAL

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Abstract

Background

Acute fibrinous and organizing pneumonia (AFOP) after lung transplantation is associated with a rapid decline in pulmonary function. However, the relation with chronic lung allograft dysfunction (CLAD) remains unclear. We investigated the association between detection of AFOP in lung allograft biopsies with clinically important endpoints.

Methods

We reviewed lung allograft biopsies from 468 patients who underwent lung transplantation at the University Hospitals Leuven (2011-2017). AFOP was categorized as *early* new-onset (≤90 days post-transplant) or *late* new-onset (>90 days post-transplant); and associated with CLAD-free survival, graft survival, donor-specific antibodies, airway and blood eosinophilia.

Results

Early and *late* AFOP was detected in 24 (5%) and 30 (6%) patients, respectively. CLAD-free survival was significantly lower in patients with *late* AFOP (median survival 2.42y, p<0.0001) compared to patients with *early* or *without* AFOP and specifically associated with development of restrictive allograft syndrome (OR: 28.57; CI [11.34 – 67.88], p<0.0001). Similarly, graft survival was significantly lower in patients with *late* AFOP (median survival 4.39y, p<0.0001) compared to patients with *early* AFOP or without AFOP. L*ate* AFOP was furthermore associated with detection of circulating donor-specific antibodies (OR: 4.75, CI [2.17-10.60], p=0.0004) compared to patients with *early* or *without* AFOP; and elevated airway and blood eosinophilia (p=0.043 and p=0.045, respectively) compared to *early* AFOP patients.

Conclusions

Late new-onset AFOP is associated with a worse prognosis and high risk of CLAD development, specifically restrictive allograft syndrome. Our findings indicate that *late* new-onset AFOP might play a role in the early pathogenesis of restrictive allograft syndrome.

Introduction

Lung transplantation is an accepted treatment for patients with various chronic end-stage lung diseases. However, long-term outcome after lung transplantation is still hampered by development of chronic lung allograft dysfunction (CLAD) in approximately 50% of patients 5 years after lung transplantation (1). CLAD patients represent a heterogeneous population: clinically, radiologically, and based on histopathologic findings. In general, two main clinical phenotypes of CLAD have been defined: bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome (RAS) (2,3). The first is defined by a progressive and irreversible obstructive pulmonary function defect, the latter by a restrictive pulmonary function defect, characterized by persistent pleuroparenchymal opacities on computed tomography scan and portending a poor outcome (3,4).

Acute fibrinous and organizing pneumonia (AFOP) was initially described in 2002 by Beasley at al. as a distinct histologic pattern associated with acute lung injury (5). It is characterized by the presence of prominent intra-alveolar fibrin deposition and organizing pneumonia, different from other histologic patterns, such as diffuse alveolar damage (DAD) or eosinophilic pneumonia. Two distinct forms of disease progression and clinical outcome were described: a fulminant form with rapid disease progression leading to death and a subacute form with a better prognosis (5).

In 2013, Paraskeva et al. identified AFOP as a novel entity in 22/194 (11%) lung transplant recipients, invariably associated with a rapid decline in respiratory function and death after a median time of 101 days (6). The subacute form described by Beasley et al. was not seen in any of these patients. In addition, Paraskeva et al. did not detect histologic evidence of AFOP in patients diagnosed with a "RAS-like" syndrome, whereas histopathologic analysis of explant lungs recently revealed presence of AFOP in approximately 50% of clinically defined end-stage RAS patients (7). Therefore, the clinical behavior of AFOP and its relation to RAS in lung transplant recipients remains to be elucidated.

In addition, the importance of the time-of-onset of AFOP on outcome has not been assessed. For other injury patterns, such as DAD and organizing pneumonia, an important time-dependent effect

of the time-of-onset of injury on CLAD-free and graft survival was observed (8,9). Specifically *late* new-onset DAD (>3 months after transplantation) has been associated with an increased risk of subsequent RAS development, whereas *early* DAD was associated with early mortality and BOS (8).

The purpose of this study is therefore to investigate the relationship between AFOP in lung allograft biopsies on the one hand and functional and clinical relevant outcomes on the other hand. We hypothesized that *late* new-onset AFOP may be associated with development of RAS and worse graft survival.

Materials and methods

Patient selection, histopathologic and radiologic assessment

The study included all patients who underwent lung transplantation at the University Hospitals Leuven (Leuven, Belgium) between January 2011 and December 2017. Retransplantation was considered as a separate event in our analysis. Follow-up was censored on September 1st, 2018. All diagnostic biopsies (transbronchial biopsies (TBB), computed-tomography guided biopsies and surgical biopsies) were initially evaluated by a single experienced lung pathologist who formed a systematic and detailed descriptive pathology report. For the current study, the pathology reports of all diagnostic biopsies, both surveillance and indication biopsies (e.g. suspicion of infection or rejection), were reviewed. Hematoxylin-eosin stained slides and staining's for micro-organisms from all initial reports consistent with AFOP were re-evaluated by two experienced lung pathologists blinded for all patient data, until consensus was reached. If multiple positive biopsies were available for a single patient, the first biopsy displaying AFOP was considered as the time of new-onset AFOP. Based on the date of new-onset AFOP, *early* (≤ 90 days post lung transplantation (post-Ltx)) vs. *late* new-onset (> 90 days post-LTx) AFOP was defined, by applying the same cut-off of 90 days previously used to investigate the importance of the time-of-onset of histological injury on outcome *(Figure 1)* (8). If available, explant lung biopsies (obtained at autopsy or retransplantation) from patients with graft loss were also re-evaluated. AFOP was diagnosed according to the criteria proposed by Beasley

et al. with presence of at least 2 major features (i.e. prominent intra-alveolar fibrin, organizing pneumonia, and patchy distribution), without evidence of hyaline membranes, eosinophilic infiltration or granulomatous inflammation (5). Chest computed tomography (CT) imaging at diagnosis of AFOP was reviewed by an experienced thoracic radiologist, blinded for the study design. The transplant monitoring schedule, histopathologic, and radiologic assessment are described in detail in the *online supplementary appendix*.

Laboratory results

Broncho-alveolar lavage (BAL) was routinely performed during bronchoscopy by instillation of 2x50 ml of saline, as previously described (10). Similarly, peripheral blood samples were collected at time of bronchoscopy. Total and differential white blood cell (WBC) counts, C-reactive protein (CRP), and presence of persistent *de novo* anti-human leukocyte antigen (HLA) donor-specific antibodies (DSAs) were assessed, as previously reported (11).

Graft loss and CLAD diagnosis

Graft loss was defined as death (i.e. all-cause mortality) or retransplantation. CLAD was defined as a persistent (>3 months) forced expiratory volume in one second (FEV_1) decline of at least 20% compared to the mean of the 2 best post-operative FEV_1 measurements obtained >3 weeks apart (follow-up until September 2018), in absence of another cause (4). RAS was defined by an additional >10% decline in total lung capacity (TLC) and/or >20% drop in FVC and evidence of persistent radiological opacities (3). CLAD-free survival was defined as the time between transplant and the initial onset of >20% FEV1 decline.

Survival sub-analysis

A sub-analysis was performed to define whether detection of *late* AFOP was an independent poor prognostic factor. Therefore, we compared CLAD-free and graft survival in *late* AFOP patients, patients without indication biopsy (i.e. a for-cause biopsy > 90 days after transplantation), patients

with an indication biopsy (n ≥ 1) but with normal findings and patients with an indication biopsy (n ≥ 1) with abnormal findings (i.e. presence of acute rejection or infection, but absence of AFOP) (patient characteristics are provided in *Table S3*). Patients with graft survival of ≤90 days post lung transplantation were excluded for this survival sub-analysis because presence of an indication biopsy was de facto not assessable.

Data expression and ethical considerations

Kaplan-Meier analysis and log-rank tests were used for survival analysis; the relationship between AFOP, RAS and DSAs, was analyzed using Fisher's exact test. BAL and peripheral blood counts were compared using Mann Whitney test. Adjusted CLAD-free and graft survival analysis was performed using a Cox proportional hazards model adjusting for native lung disease (emphysema, interstitial lung disease, cystic fibrosis or bronchiectasis, other), age at transplant, sex, type of transplant, episodes of acute rejection, episodes of lymphocytic bronchiolitis, histologic evidence of infection and CMV infection, epoch (year of LTx), and occurrence of de novo persistent DSAs. GraphPad statistical software (Prism, version 7.01, San Diego CA, USA) and SAS (SAS Institute, version 9.3, Cary, NC) was used for all analyses. A p value of <0.05 was considered significant. This retrospective study was approved by the local ethics committee (S52174).

Results

The majority of all 468 included patients never displayed AFOP on diagnostic biopsies (n=414, 89%), whereas 24 (5%) patients presented with *early* new-onset AFOP; and 30 (6%) patients with *late* new-onset AFOP (*Figure 2*). Patient characteristics are summarized in *Table 1* and an overview of histopathologic findings in diagnostic biopsies is provided in *Table 2*. Treatment regimens of AFOP patients are described in the *online supplementary appendix*.

CLAD-free survival

CLAD-free survival was significantly lower in patients with *late* AFOP (median survival 2.42y, p<0.0001) compared to patients without AFOP or patients with *early* AFOP *(Figure 3, left)*. Eighty-five (21%) patients without AFOP developed CLAD (RAS, n=14 (16%); BOS, n=71 (84%)), 4 (17%) patients with *early* AFOP developed CLAD (RAS, n=1 (25%); BOS, n=3 (75%)), and 19 (63%) patients with *late* AFOP developed CLAD (RAS, n=15 (79%); BOS, n=4 (21%)) *(Table 3)*. RAS was clinically diagnosed using a >10% TLC decline in 25 of 30 (83%) RAS patients and >20% FVC decline in the remaining patients.

Patients with *late* AFOP were more likely to develop RAS, compared to patients without AFOP (OR: 28.57; CI [11.34 – 67.88], p<0.0001). Interestingly, 3 patients with *late* AFOP progressed from a BOS to a RAS phenotype of CLAD shortly following the detection of *late* AFOP and were considered as RAS patients for further analysis. Median interval between clinical RAS diagnosis and detection of *late* AFOP was -16 days (IQR: -72 – 14). In contrast, *early* AFOP demonstrated no significant correlation with later RAS development (OR: 1.24; CI [0.11 – 7.19], p=0.58).

Graft survival

Graft survival was significantly lower in patients who developed *late* AFOP (median survival 4.39y, p<0.0001) compared to patients without AFOP or patients with *early* AFOP *(Figure 3, right)*. Graft loss occurred in 74 (18%) patients without AFOP (death, n=67; retransplantation, n=7) and in 2 (8%)

patients with *early* AFOP (death, n=1; retransplantation, n=1). Graft loss was noted in 17 (57%) *late* AFOP patients (death, n=13; retransplantation, n=4), mostly due to CLAD (n=15, specifically RAS, n=13), humoral rejection (n=1), or sepsis (n=1).

Histopathologic findings in explant lungs

Explant lung biopsies of 11/17 (65%) *late* fibrin/OP patients with graft loss were available. Interstitial fibrotic changes, consistent with RAS, were present in 10/11 (91%) patients. A non-specific interstitial pattern of fibrosis was present in 3/11 (27%) patients, AFOP in 3/11 (27%) patients, and pleuroparenchymal fibro-elastosis in 4/11 (36%) patients. In addition, bronchiolitis obliterans lesions were detected in 10/11 (91%) patients. Histopathologic findings in *early* AFOP explant lungs (n=2) are described in the *online supplementary appendix*.

Radiologic findings

Chest CT imaging was available at AFOP diagnosis for all 54 AFOP patients (*Table 2*). *Late* AFOP patients presented with radiological opacities in 27/30 (90%) patients, mostly diffusely present in 23 (77%) patients. *Late* AFOP patients had significantly more air trapping and less pleural effusions compared to *early* AFOP patients (p=0.006 and p=0.01, respectively).

Presence of DSAs

Occurrence of persistent de novo DSAs was more prevalent in patients with *late* AFOP (n=11, 37%) compared to patients without AFOP (n=45, 11%) or patients with *early* AFOP (n=2, 8%) (p=0.024) (*Table 3*). *Late* AFOP was significantly associated with the presence of DSAs (OR: 4.75, CI [2.17-10.60], p=0.0004), compared to patients without AFOP. Occurrence of DSAs in patients with *late* AFOP did not significantly impact graft survival (p=0.058), compared to patients with *late* AFOP without DSAs. Similarly, detection of DSAs had no impact on CLAD-free survival in *late* AFOP patients (p=0.15). *Early* AFOP showed no correlation with the presence of DSAs (OR: 0.75, CI [0.17 – 2.98], p>0.99).

BAL and peripheral blood

BAL fluid differential cell counts were obtained in 19 (79%) of 24 patients with *early* AFOP and in 23 (77%) of 30 patients with *late* AFOP. BAL eosinophilia was significantly higher in patients with *late* AFOP (median 0.5%; IQR [0 – 5.20]), compared to patients with *early* AFOP (median 0%; interquartile range (IQR) [0 – 0.40]) (p=0.043). Seven (23%) patients with *late* AFOP had a concomitant BAL eosinophilia of >2% (i.e. the upper limit of normal in our laboratory), whereas only 1 (2%) patient with *early* AFOP had a BAL eosinophilia of >2% (p = 0.054). Analysis of BAL fluid revealed no difference in infection rates (p=0.74, details in *online supplementary appendix*).

Blood leukocyte differentiation was available for 18 (75%) of 24 patients with *early* AFOP and 29 (97%) of 30 patients with *late* AFOP. Blood eosinophilia was significantly higher in patients with *late* AFOP (median 100/µL; IQR [0 – 200]) compared to patients with *early* AFOP (median 0/µL; IQR [0 – 100]) (p=0.045). Other BAL and peripheral blood measurements did not significantly differ between both groups *(Table S1)*.

Adjusted and survival sub-analysis

Multivariate analysis demonstrated that detection of *late* AFOP was an independent risk factor for both CLAD-free survival (hazard ratio [HR] 3.11, [CI] 1.76 to 5.27, p<0.0001) and graft survival ([HR] 3.03, [CI] 1.71 to 5.36, $p = 0.0001$) (*Table S2*). In addition, survival sub-analysis revealed that detection of *late* AFOP portended a significantly lower CLAD-free and graft survival compared to patients with an abnormal indication biopsy, but without AFOP (p = 0.035; p = 0.0003, respectively) *(Figure 4).* (patient characteristics and information on indication biopsies are provided in *Table S3 and Table S4*). A visual representation of the relation between the time to new-onset AFOP, detection of DSAs, CLAD-free and graft survival is provided in *Figure S1 and S2*. CLAD-free and graft survival analysis applying different cut-offs for *early* vs *late* AFOP (i.e. 3m, 6m, 9m, 12m, 18m, 24m) is provided in *Figure S3* and *S4*, all confirming the inferior outcome of *late* AFOP."

Discussion

We investigated the association between AFOP in diagnostic biopsies and different functional and clinical parameters in diagnostic biopsies in a large cohort of lung transplant patients. The main findings of this study are (i) a lower CLAD-free and graft survival in patients with *late* AFOP, whereas *early* AFOP demonstrated no correlation with outcome; (ii) a strong association between *late* AFOP and development of RAS; and (iii) a link between *late* AFOP and DSAs.

Paraskeva et al. previously demonstrated that detection of AFOP in lung transplant patients was invariably associated with poor outcome, and reported a median survival of only 101 days (6). Our findings do not completely support this observation, as detection of *early* new-onset AFOP had no effect on outcome in our patient cohort. Detection of *late* new-onset AFOP strongly correlated with poor outcome, and patients were at high risk of CLAD development, particularly RAS. The possible link between AFOP and RAS has previously been reported by our research group based on histopathologic analysis of explant lungs, which revealed the presence of AFOP in approximately 50% of clinically identified RAS patients (7). In contrast, Paraskeva et al. did not observe histologic changes consistent with AFOP in patients with a "RAS-like" clinical phenotype (but without TLC confirmation) (6).

We found that first detection of *late* AFOP roughly accompanied the clinical and radiological diagnosis of RAS, which might support the hypothesis that AFOP represents an early histopathologic hallmark in the pathogenesis of RAS development, leading to rapid decline in respiratory function and death in case of a fulminant course, or to CLAD in patients surviving the acute onset.

Our results are in line with previous observations demonstrating an association between specific histopathologic patterns (i.e. *late* new-onset DAD and organizing pneumonia) and RAS development (8,9). We found no association between *early* AFOP and CLAD-free or graft survival; which indicates a time-dependent effect of AFOP onset on CLAD-free survival. Indeed, it seems that *early* AFOP mostly represents a transient process and resolves without excessive CLAD development. However, as *early*

and *late* AFOP are histologically indistinguishable, the prognosis might be dependent on the underlying cause of AFOP.

AFOP has a typical patchy distribution and definite exclusion of AFOP might be difficult based on a small-sized TBB. In the context of a high clinical suspicion, a negative TBB may therefore prompt further investigation (e.g. additional TBB sampling). In addition, a definite diagnosis of AFOP based on a TBB can be challenging as other histologic patterns (e.g. DAD, eosinophilic pneumonia) may resemble the histopathologic changes seen in AFOP (5).

We found a significant association between *late* AFOP and the presence of persistent *de novo* DSAs. Interestingly, *late* AFOP patients exclusively developed DSAs against HLA type II DQ antigens. Persistent *de novo* DSAs, and specifically DSA-DQ antibodies, have been associated with a higher risk for CLAD and RAS in particular (11,12). The underlying pathophysiology and their potential role in the causative pathway of RAS remain unclear, but these results are in line with a previous report that demonstrated a link between antibody-mediated rejection and RAS (13). Our current findings support this hypothesis and might point towards a pivotal role for intra-alveolar fibrin deposition. We previously postulated that microvascular injury, at least partly triggered by antibody mediated rejection, might be the initial event leading to capillary leakage and intra-alveolar fibrin deposition (7) (*Figure S5*). In a next phase, there seems to be an inefficient clearing of intra-alveolar fibrin, which then forms loosely textured fibrin balls (14). Next, fibroblasts might proliferate and infiltrate the fibrin balls, and result in the typical AFOP pattern. Ultimately, this fibrotic response might lead to a pattern of (sub)pleural and septal fibrosis, characteristic of RAS. In addition, as bronchiolitis obliterans lesions were found in the vast majority of available explant lungs from *late* AFOP patients, an unknown pathogenic link between AFOP and bronchiolitis obliterans lesions might be present.

We observed higher eosinophil levels in blood and BAL samples in *late* AFOP patients, compared to *early* AFOP patients. However, we did not observe increased tissue eosinophilia in AFOP biopsies, in which case a concomitant infectious disease or eosinophilic pneumonia should be ruled out. We

previously demonstrated that a BAL eosinophilia of ≥2% predisposed to later RAS development (15), as well as to lower survival after RAS diagnosis (16). Eosinophils may play a role in the pathophysiology of CLAD, although the underlying mechanisms remain unknown.

Our study has several limitations. First, patient selection was performed retrospectively based on revision of the pathology report. Second, this study is based on data from single-center patients, and although we report a large patient cohort, extrapolation of these results to draw general conclusions about lung transplant patients might be restricted. Third, blood and BAL differential cell counts were not available for all patients with AFOP, due to technical difficulties obtaining adequate samples in clinically unstable patients. Furthermore, blood and BAL cell counts could not be compared to patients without AFOP, as no suitable reference time-point could be defined in patients without AFOP.

In conclusion, we demonstrate that *late* new-onset AFOP is associated with lower CLAD-free and graft survival, and more specifically development of RAS. We believe that these findings provide evidence that might suggest that AFOP is a key histopathologic feature in the early pathogenesis of RAS. Further characterization of the pathogenic mechanisms underlying AFOP and RAS development should contribute to a better understanding of the complex relation between AFOP and RAS.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

Figure legends

Figure 1. Flowchart of patient selection.

Figure 2. Histology of acute fibrinous and organizing pneumonia (AFOP) (Hematoxylin-Eosin stain, x20). Prominent loose intra-alveolar fibrin balls are present within the alveolar spaces (arrow) mixed with fibroblasts.

Figure 3. *Left.* Kaplan-Meier curve illustrating CLAD-free survival post-LTx. CLAD-free survival is significantly worse in patients with *late* AFOP compared to patients with *early* AFOP, or patients without AFOP ($p<0.0001$). *Right.* Kaplan-Meier curve illustrating graft survival post-LTx. Graft survival is significantly worse in patients with *late* AFOP compared to patients with *early* AFOP, or patients without AFOP ($p<0.0001$). AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

Figure 4. *Left.* Kaplan-Meier curve illustrating CLAD-free survival post-LTx. *Right.* Kaplan-Meier curve illustrating graft survival post-LTx. CLAD-free survival and graft survival are significantly worse in patients with *late* AFOP compared to patients with an indication biopsy with abnormality, patients with an indication biopsy without abnormality, or patients without indication biopsy ($p < 0.0001$; $p < 0.0001$, respectively). Patients with a graft survival of ≤90 days post lung transplantation (n=18) were excluded because presence of an indication biopsy (i.e. a for-cause biopsy >90 days after transplantation) was de facto not assessable. AFOP: acute fibrinous and organizing pneumonia, CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

Table legends

Table 1. 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. 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 2. Data are shown as n, n (%) or median (interquartile range). Histopathologic and radiologic findings in the *early* and *late* AFOP group were compared using Fisher's exact test; time of biopsy was compared using Mann Whitney test. AFOP: acute fibrinous and organizing pneumonia; LTx: lung transplantation; TBB: transbronchial biopsy; CT: computed tomography; AR: acute rejection; LB: lymphocytic bronchiolitis; RBCs: red blood cells; GGOs: ground-glass-opacities.

Table 3. Data are shown as n, n (%) or median (interquartile range). Groups were compared using Chi Square test; time to DSAs was compared using Kruskal-Wallis test. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; BOS: bronchiolitis obliterans syndrome; RAS: restrictive allograft syndrome; DSA: donor-specific antibodies; HLA: human leukocyte antigen; LTx: lung transplantation. (*) DSA categories are mutually exclusive.

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Table 1

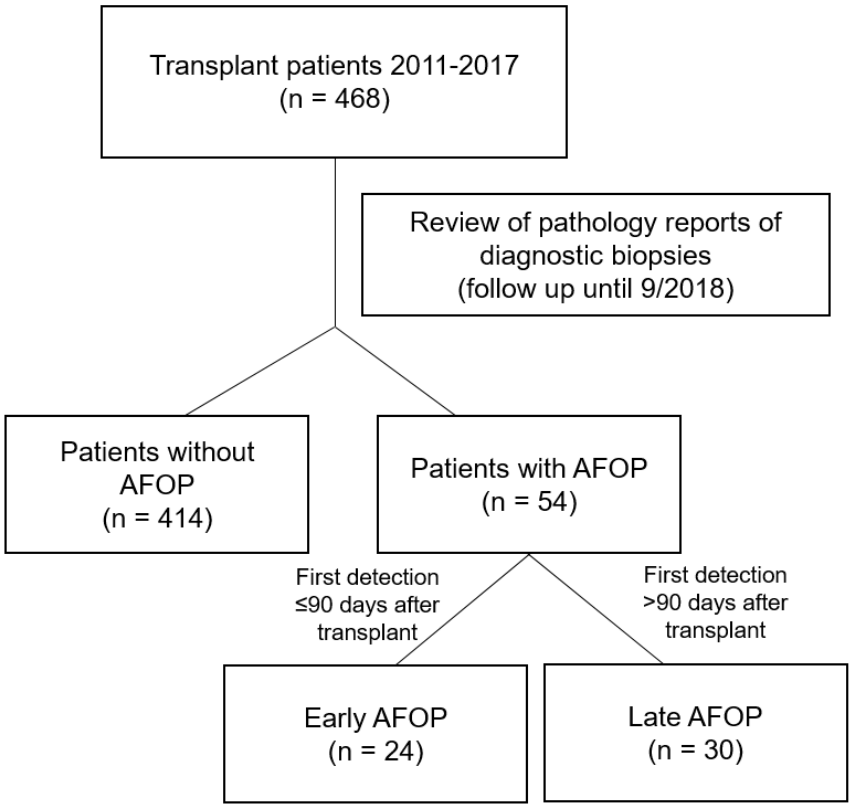
Table 1. Patient characteristics					
	Total	**No AFOP**	**Early AFOP**	**Late AFOP**	**p value**
Patients, N (%)	468	414 (89)	24 (5)	30 (6)	
Age at transplant (years)	57 (45-61)	57 (47-61)	47 (35-54)	55 (44-62)	**0.01**
Male, N (%)	235 (50)	203 (49)	14 (58)	18 (60)	0.37
Underlying disease, N (%)					0.18
Emphysema	251 (54)	226 (55)	8 (33)	17 (57)	
ILD	92 (20)	81 (20)	4 (17)	7 (23)	
CF or BRECT	74 (16)	60 (14)	9 (38)	5 (17)	
Redo transplant	31 (7)	28 (7)	3 (13)	0	
PHT or Eisenmenger	17 (4)	16 (4)	0	1 (3)	
Other	3 (0.6)	3 (0.7)	0	0	
Type of transplant, N (%)					0.32
SSLTx	451(96)	400 (97)	22 (92)	29 (97)	
SSLTx + LiTx	7 (1)	6 (1)	1 (4)	0	
HLTx	6 (1)	5 (1)	0	1 (3)	
SSLTx + KiTx	2 (0.4)	1 (0.2)	1 (4)	0	
SLTx	1 (0.2)	1 (0.2)	0	0	
HLTx + LiTx	1 (0.2)	1 (0.2)	0	0	

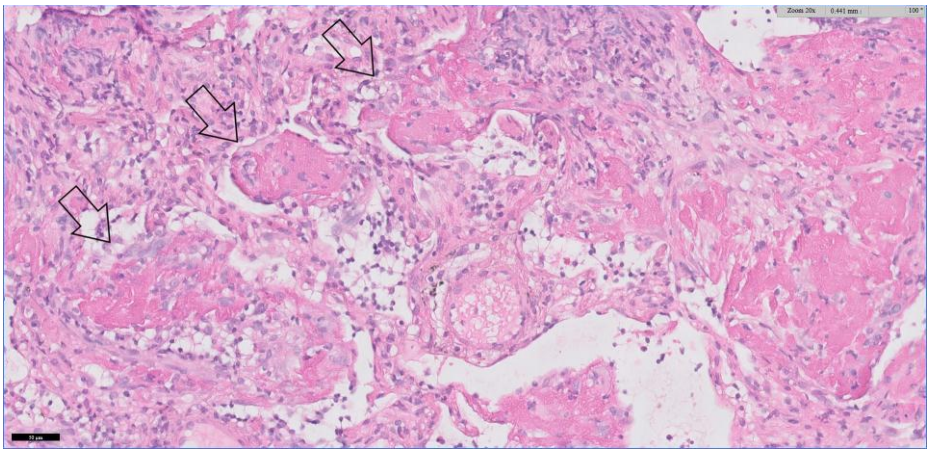
Table 2

Table 2. Histopathologic and radiologic findings			
	Early AFOP	**Late AFOP**	**p value**
Patients, N	24	30	
Time of biopsy (days post-LTx)	22 (15– 29)	694 (336 – 1205)	
Type of biopsy, N (%)			
TBB	23 (96)	29 (97)	>0.99
CT guided biopsy	0	1 (3)	>0.99
Surgical biopsy	1 (4)	0	0.44
Histopathologic findings, N (%)			
AR	7 (29)	2 (7)	0.062
LB	0	2 (7)	0.50
RBCs intra-alveolar	13 (54)	6 (20)	**0.012**
Hemosiderin laden macrophages intra-alveolar	2 (8)	4 (13)	0.68
Radiologic findings, N (%)			
Presence of radiological opacities	20 (83)	27 (90)	0.69
Nodular opacifications	6 (25)	12 (40)	0.38
GGOs	15 (63)	22 (73)	0.56
Crazy paving pattern	6 (25)	8 (27)	>0.99
Consolidation	12 (50)	14 (47)	>0.99
Location of opacities			
Diffuse	14 (58)	23 (77)	0.24
Apical only	0 (0)	1 (3)	>0.99
Basal only	6 (25)	3 (10)	0.16
Pleural effusion	19 (79)	13 (43)	**0.01**
Air trapping	2 (8)	13 (43)	**0.006**

Table 3

Table 3. CLAD incidence and presence of DSAs				
	No AFOP	**Early AFOP**	**Late AFOP**	**p value**
Patients, N	414	24	30	
CLAD, N (%)	85 (21)	4 (17)	19 (63)	**<0.0001**
BOS	71 (17)	3 (13)	4 (13)	
RAS	14 (3)	1 (4)	15 (50)	
Presence of DSAs, N (%)*	45 (11)	2 (8)	11 (37)	**0.0002**
HLA type I	4 (1)			0.54
HLA type II				
HLA type II (DQ)	27 (7)		11 (37)	**0.0022**
HLA type II (DR)	5 (1)			0.45
HLA type II (DQ + DR)	6 (1)	2 (8)		**0.0008**
HLA type II (DP)	2 (0.4)			0.74
HLA type I + type II	1 (0.2)			0.86
Time to DSAs (years' post-LTx)	1.01 (0.09 – 2.09)	0.14 (0.06-0.21)	2 (1.45-2.29)	0.13


Transplant patients 2011-2017
(n = 468)
Review of pathology reports of diagnostic biopsies
(follow up until 9/2018)
Patients without AFOP
(n = 414)
Patients with AFOP
(n = 54)
First detection ≤90 days after transplant
First detection >90 days after transplant
Early AFOP
(n = 24)
Late AFOP
(n = 30)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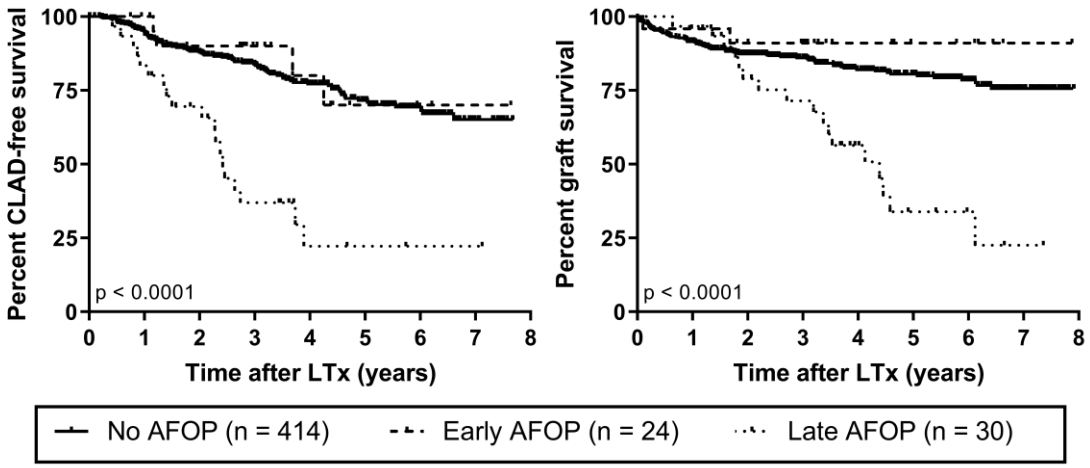
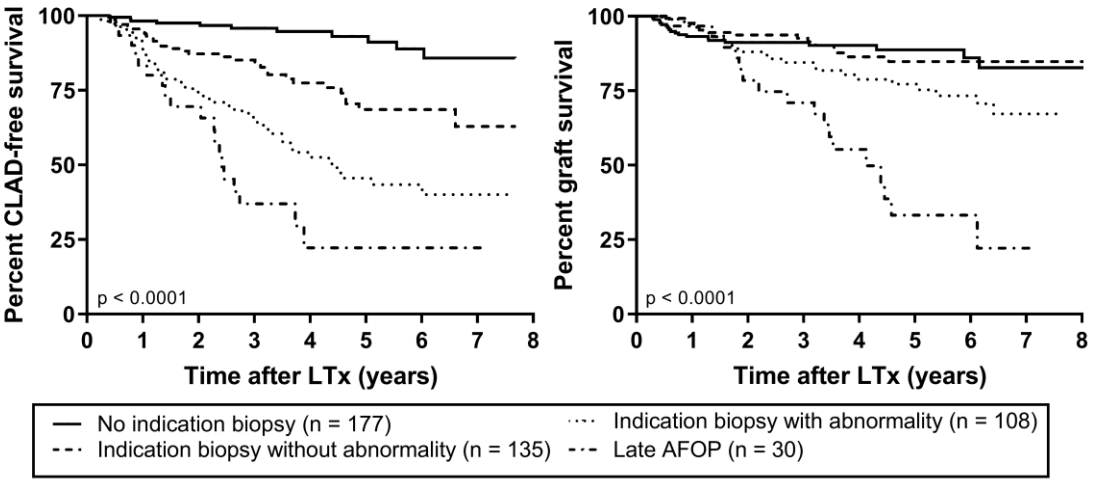
0.441 mm
100 µm


Percent CLAD-free survival
100
75
50
25
0
p < 0.0001
0 1 2 3 4 5 6 7 8
Time after LTx (years)
Percent graft survival
100
75
50
25
0
p < 0.0001
0 1 2 3 4 5 6 7 8
Time after LTx (years)
No AFOP (n = 414)
Early AFOP (n = 24)
Late AFOP (n = 30)


Percent CLAD-free survival
100
75
50
25
0
p < 0.0001
0 1 2 3 4 5 6 7 8
Time after LTx (years)
Percent graft survival
100
75
50
25
0
p < 0.0001
0 1 2 3 4 5 6 7 8
Time after LTx (years)
No indication biopsy (n = 177)
Indication biopsy with abnormality (n = 108)
Indication biopsy without abnormality (n = 135)
Late AFOP (n = 30)

ONLINE SUPPLEMENT

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

Vanstapel A., Verleden S., Weynand B. et al.

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

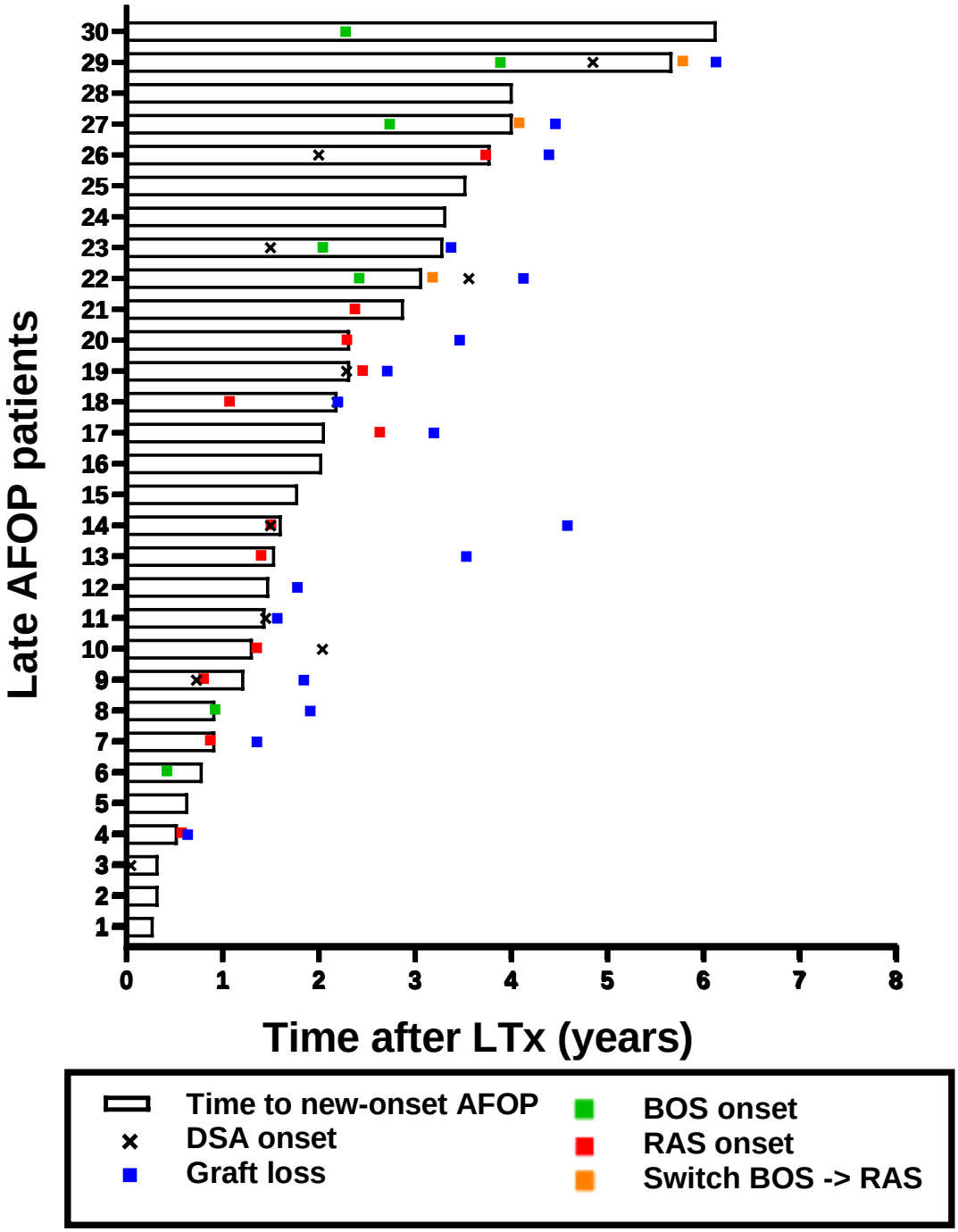


Figure S1. Timeline illustrating the relation between the time to new-onset *late* AFOP, detection of DSAs, CLAD-free and graft survival of individual *late* AFOP patients. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

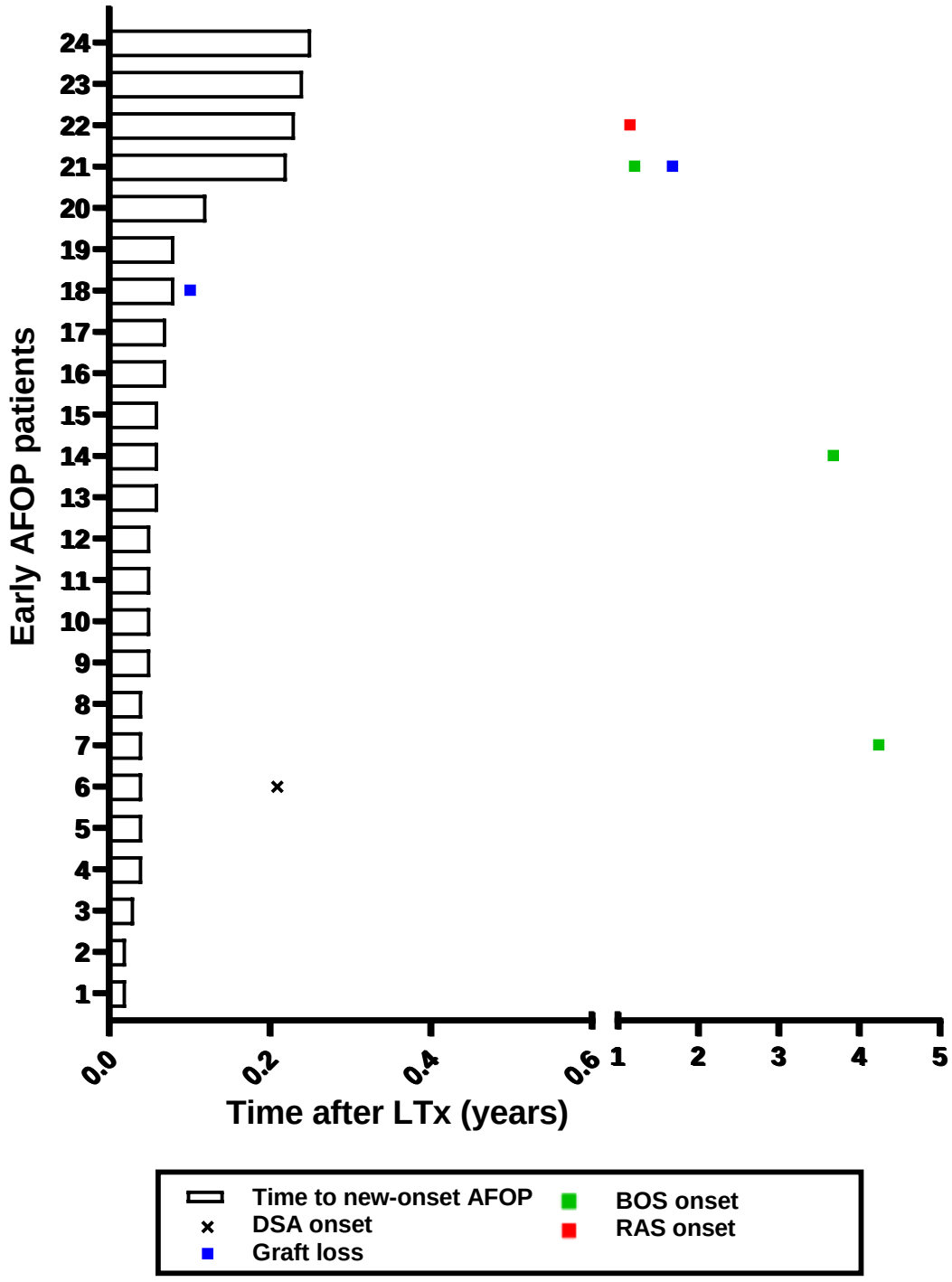


Figure S2. Timeline illustrating the relation between the time to new-onset *early* AFOP, detection of DSAs, CLAD-free and graft survival of individual *early* AFOP patients. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

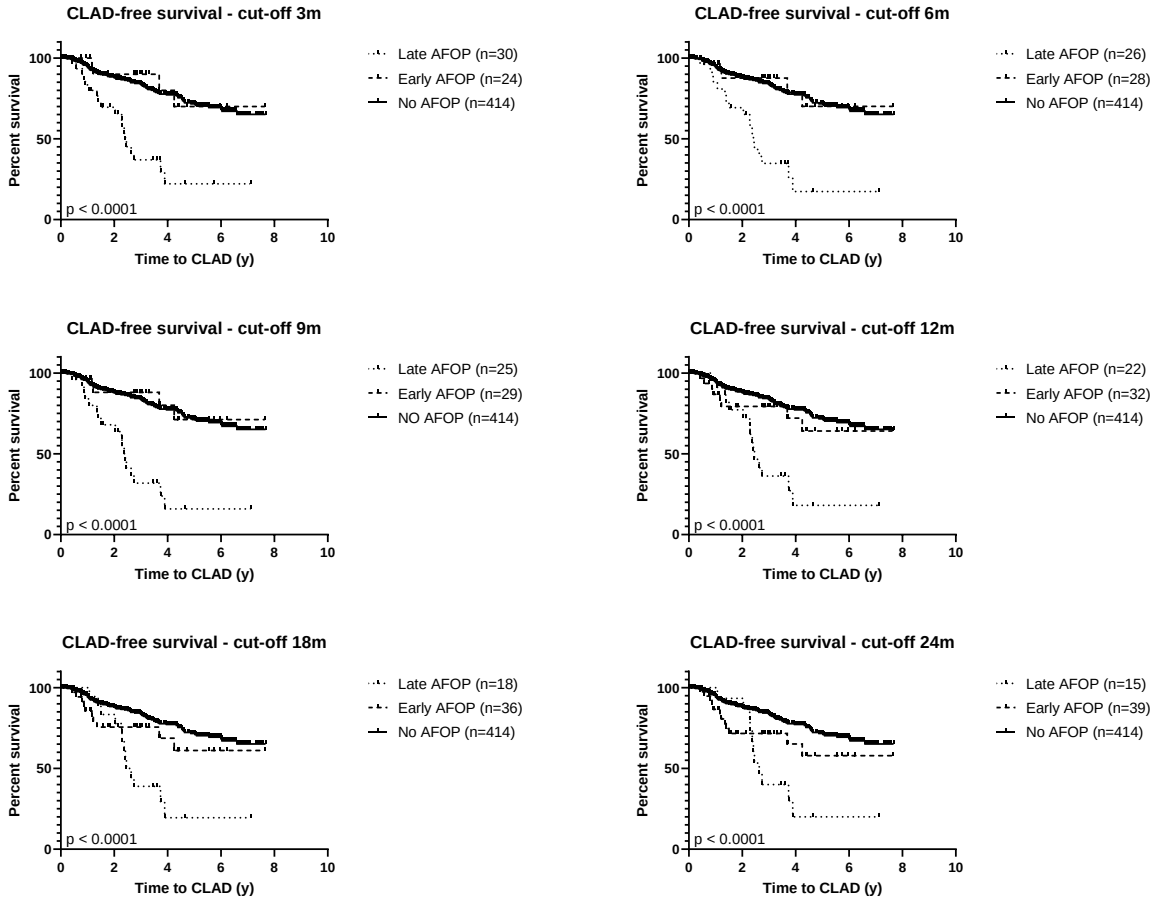


Figure S3. Kaplan-Meier curves illustrating the influence of applying several cut-offs (i.e. 3m, 6m, 9m, 12m, 18m, 24m) on CLAD-free survival. AFOP: acute fibrinous and organizing pneumonia; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

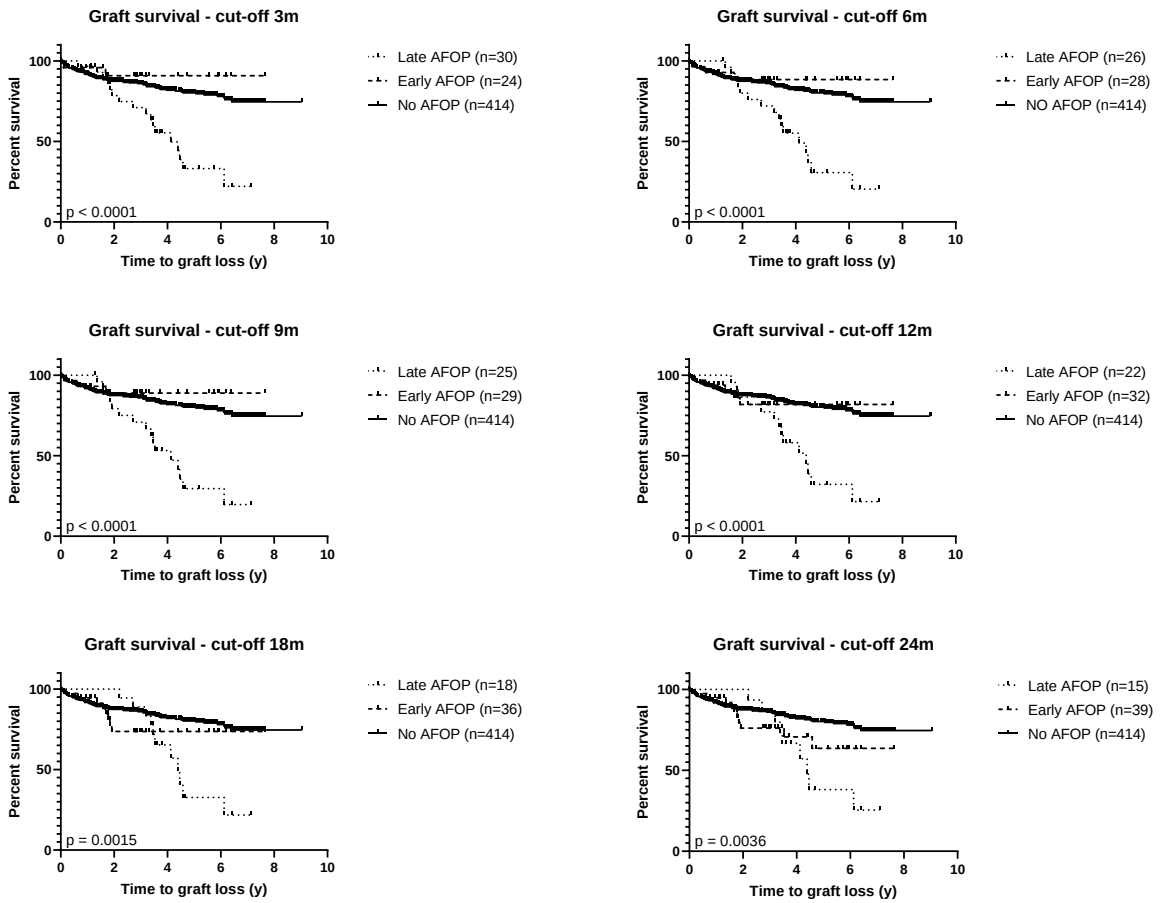


Figure S4. Kaplan-Meier curves illustrating the influence of applying several cut-offs (i.e. 3m, 6m, 9m, 12m, 18m, 24m) on graft survival. AFOP: acute fibrinous and organizing pneumonia; LTx: lung transplantation.

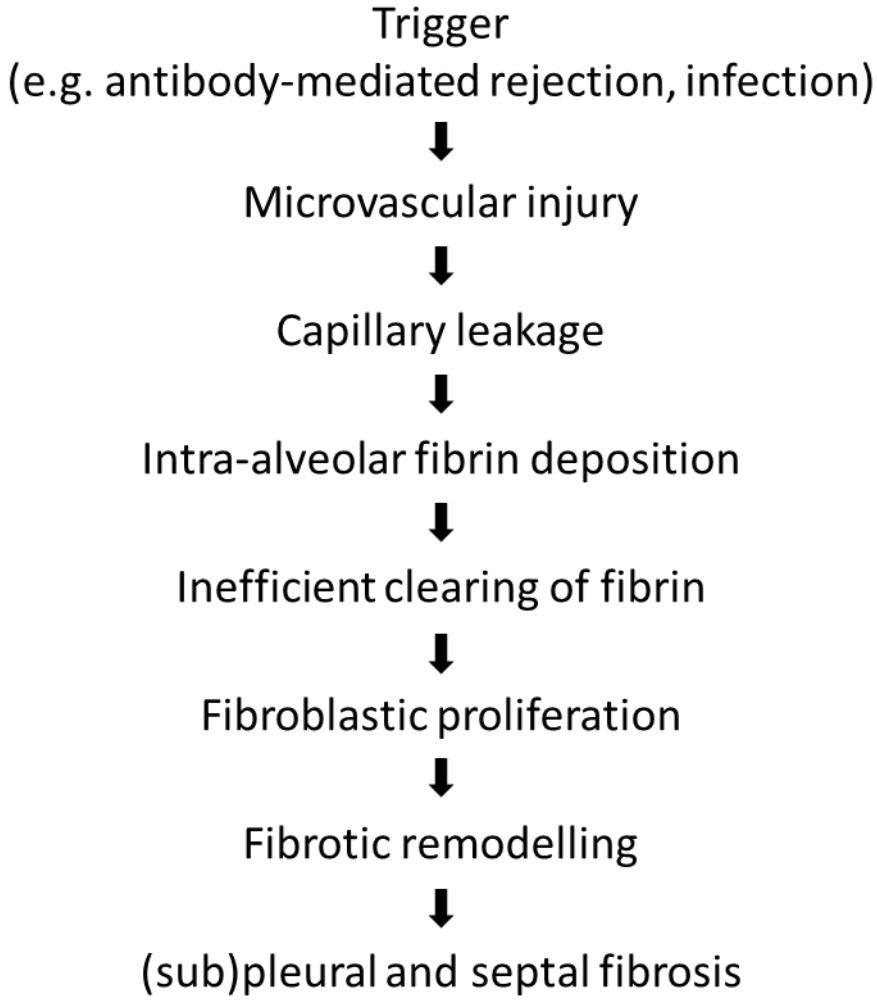


Figure S5. Proposed pathologic cascade leading to the development of restrictive allograft syndrome.

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.