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

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This study links acute fibrinous and organising pneumonia with poor outcome after lung transplantation. These findings indicate that acute fibrinous and organising pneumonia plays a role in the pathogenesis of restrictive allograft syndrome. https://bit.ly/3aof9n9

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ABSTRACT Acute fibrinous and organising 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.

We reviewed lung allograft biopsies from 468 patients who underwent lung transplantation at the University Hospitals Leuven (2011–2017). AFOP was categorised 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.

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 2.42 years; p<0.0001) compared with patients with early or without AFOP and specifically associated with development of restrictive allograft syndrome (OR 28.57, 95% CI 11.34–67.88; p<0.0001). Similarly, graft survival was significantly lower in patients with late AFOP (median survival 4.39 years; p<0.0001) compared with patients with early AFOP or without AFOP. Late AFOP was furthermore associated with detection of circulating donor-specific antibodies (OR 4.75, 95% CI 2.17–10.60; p=0.0004) compared with patients with early or without AFOP, and elevated airway and blood eosinophilia (p=0.043 and p=0.045, respectively) compared with early AFOP patients.

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.

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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 histopathological 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, characterised by persistent pleuroparenchymal opacities on computed tomography scan and portending a poor outcome [3, 4].

Acute fibrinous and organising pneumonia (AFOP) was initially described in 2002 by Beasley *et al.* [5] as a distinct histological pattern associated with acute lung injury. It is characterised by the presence of prominent intra-alveolar fibrin deposition and organising pneumonia, different from other histological 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.* [6] identified AFOP as a novel entity in 22 out of 194 (11%) lung transplant recipients, invariably associated with a rapid decline in respiratory function and death after a median time of 101 days. The subacute form described by Beasley *et al.* [5] was not seen in any of these patients. In addition, Paraskeva *et al.* [6] did not detect histological evidence of AFOP in patients diagnosed with a "RAS-like" syndrome, whereas histopathological analysis of explant lungs recently revealed presence of AFOP in approximately 50% of clinically defined end-stage RAS patients [7]. Therefore, the clinical behaviour 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 organising 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 functionally and clinically relevant outcomes on the other hand. We hypothesised that late new-onset AFOP may be associated with development of RAS and worse graft survival.

Materials and methods

Patient selection, histopathological and radiological 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 1, 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. Haematoxylin–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 ($\leqslant$ 90 days post lung transplantation (post-Ltx)) *versus* 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.* [5] with presence of at least two major features (*i.e.* prominent intra-alveolar fibrin, organising pneumonia and patchy distribution), without evidence of hyaline membranes, eosinophilic infiltration or granulomatous inflammation [5]. Chest computed tomography imaging at diagnosis of AFOP was reviewed by an experienced thoracic radiologist, blinded for the study design. The transplant monitoring schedule, histopathological and radiological assessment are described in detail in the supplementary material.

Laboratory results

Broncho-alveolar lavage (BAL) was routinely performed during bronchoscopy by instillation of 2×50 mL of saline, as previously described [10]. Similarly, peripheral blood samples were collected at time of

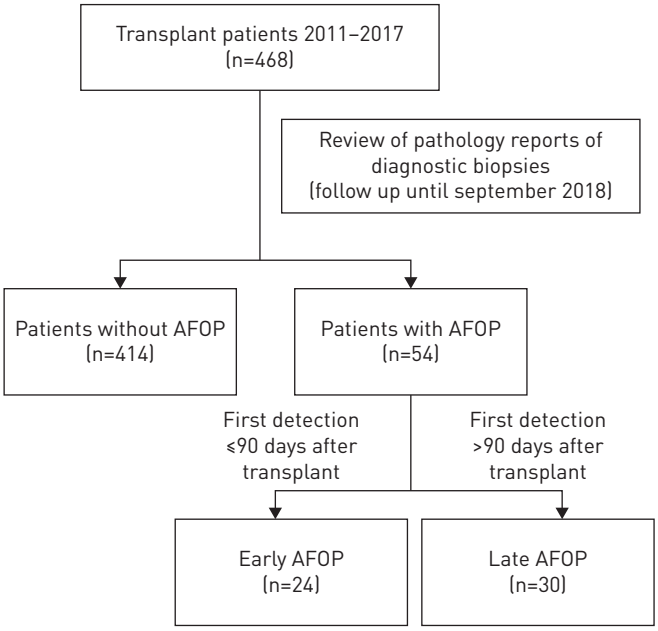

FIGURE 1 Flowchart of patient selection. AFOP: acute fibrinous and organising pneumonia.

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 1 s (FEV_1) decline of at least 20% compared to the mean of the two 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 forced vital capacity (FVC) and evidence of persistent radiological opacities [3]. CLAD-free survival was defined as the time between transplant and the initial onset of >20% FEV_1 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 post-LTx), 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-LTx 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 analysed 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, bronchiectasis or other), age at transplant, sex, type of transplant, episodes of acute rejection, episodes of lymphocytic bronchiolitis, histological 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 <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 summarised in table 1 and an overview of histopathologic

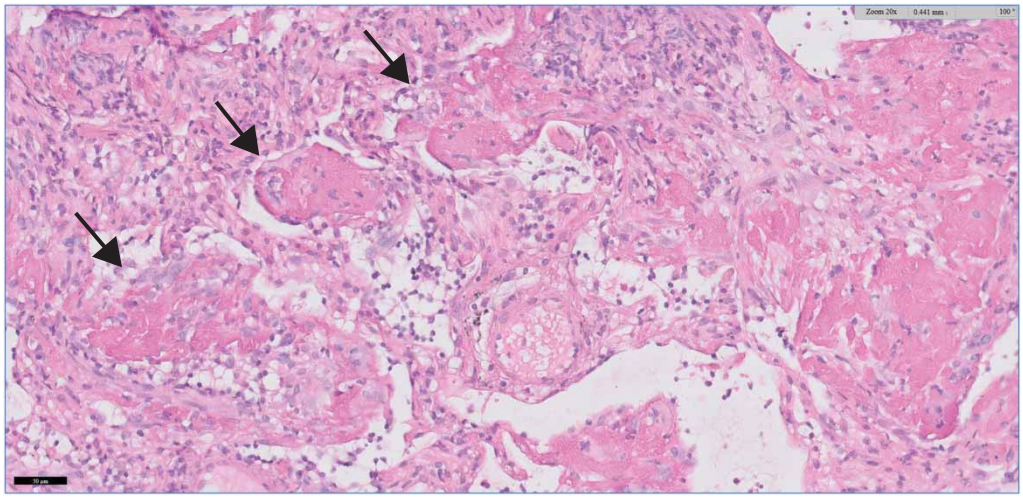

FIGURE 2 Histology of acute fibrinous and organising pneumonia (Haematoxylin–Eosin stain, ×20). Prominent loose intra-alveolar fibrin balls are present within the alveolar spaces (arrow) mixed with fibroblasts. Scale bar=50 μm.

findings in diagnostic biopsies is provided in table 2. Treatment regimens of AFOP patients are described in the supplementary material.

CLAD-free survival

CLAD-free survival was significantly lower in patients with late AFOP (median survival 2.42 years; p<0.0001) compared with patients without AFOP or patients with early AFOP (figure 3a). 85 (21%) patients without AFOP developed CLAD (RAS, n=14 (16%); BOS, n=71 (84%)), four (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 out of 30 (83%) RAS patients and >20% FVC decline in the remaining patients.

TABLE 1 Patient characteristics

	Total	No AFOP	Early AFOP	Late AFOP	p-value
Patients	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	235 (50)	203 (49)	14 (58)	18 (60)	0.37
Underlying disease					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					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	

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Patient characteristics were compared using Chi-squared test; age at transplant was compared using Kruskal–Wallis test. AFOP: acute fibrinous and organising 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. Bold indicates statistical significance (p<0.05).

TABLE 2 Histopathological and radiological findings

	Early AFOP	Late AFOP	p-value
Patients	24	30	
Time of biopsy post-LTx days	22 (15– 29)	694 (336–1205)	
Type of biopsy			
TBB	23 (96)	29 (97)	>0.99
CT-guided biopsy	0	1 (3)	>0.99
Surgical biopsy	1 (4)	0	0.44
Histopathological findings			
AR	7 (29)	2 (7)	0.062
LB	0	2 (7)	0.50
RBCs intra-alveolar	13 (54)	6 (20)	**0.012**
Haemosiderin-laden macrophages intra-alveolar	2 (8)	4 (13)	0.68
Radiological findings			
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**

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. Histopathological and radiological findings in the early and late acute fibrinous and organising pneumonia (AFOP) group were compared using Fisher's exact test; time of biopsy was compared using Mann–Whitney test. LTx: lung transplantation; TBB: transbronchial biopsy; CT: computed tomography; AR: acute rejection; LB: lymphocytic bronchiolitis; RBCs: red blood cells; GGOs: ground-glass opacities. Bold indicates statistical significance (p<0.05).

Patients with late AFOP were more likely to develop RAS, compared with patients without AFOP (OR 28.57, 95% CI 11.34–67.88; p<0.0001). Interestingly, three 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, 95% CI 0.11–7.19; p=0.58).

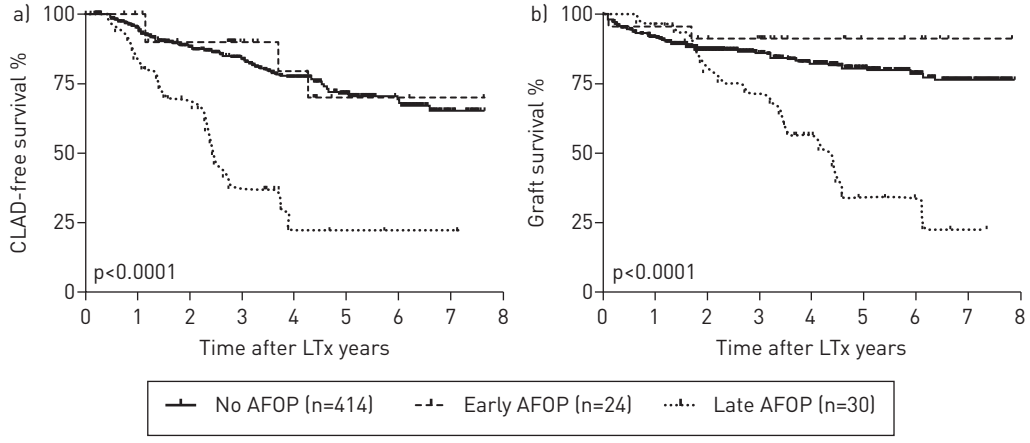

FIGURE 3 a) Kaplan–Meier curve illustrating chronic lung allograft dysfunction (CLAD)-free survival post-lung transplant (LTx). CLAD-free survival is significantly worse in patients with late acute fibrinous organising pneumonia (AFOP) compared with patients with early AFOP, or patients without AFOP (p<0.0001). b) Kaplan–Meier curve illustrating graft survival post-LTx. Graft survival is significantly worse in patients with late AFOP compared with patients with early AFOP or patients without AFOP (p<0.0001).

TABLE 3 Chronic lung allograft dysfunction (CLAD) incidence and presence of donor-specific antibodies (DSAs)

	No AFOP	Early AFOP	Late AFOP	p value
Patients	414	24	30	
CLAD	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#	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

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Groups were compared using Chi-squared test; time to DSAs was compared using Kruskal–Wallis test. AFOP: acute fibrinous and organising pneumonia; BOS: bronchiolitis obliterans syndrome; RAS: restrictive allograft syndrome; HLA: human leukocyte antigen; LTx: lung transplantation. #: DSA categories are mutually exclusive. Bold indicates significance (p<0.05).

Graft survival

Graft survival was significantly lower in patients who developed late AFOP (median survival 4.39 years; p<0.0001) compared with patients without AFOP or patients with early AFOP (figure 3b). Graft loss occurred in 74 (18%) patients without AFOP (death, n=67; retransplantation, n=7) and in two (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).

Histopathological findings in explant lungs

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

Radiological findings

Chest computed tomography imaging was available at AFOP diagnosis for all 54 AFOP patients (table 2). Late AFOP patients presented with radiological opacities in 27 (90%) out of 30 patients, mostly diffusely present in 23 (77%) patients. Late AFOP patients had significantly more air trapping and fewer 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 with 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, 95% CI 2.17–10.60; p=0.0004), compared with patients without AFOP. Occurrence of DSAs in patients with late AFOP did not significantly impact graft survival (p=0.058), compared with 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, 95% CI 0.17–2.98; p>0.99).

BAL and peripheral blood

BAL fluid differential cell counts were obtained in 19 (79%) out of 24 patients with early AFOP and in 23 (77%) out of 30 patients with late AFOP. BAL eosinophilia was significantly higher in patients with late AFOP (median 0.5%, IQR 0–5.20), compared with patients with early AFOP (median 0% 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 one (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 supplementary material).

Blood leukocyte differentiation was available for 18 (75%) out of 24 patients with early AFOP and 29 (97%) out of 30 patients with late AFOP. Blood eosinophilia was significantly higher in patients with late AFOP (median 100 cells·μL^{-1}; IQR 0–200) compared with patients with early AFOP (median 0 cells·μL^{-1}; 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 (HR 3.11, 95% CI 1.76–5.27; p<0.0001) and graft survival (HR 3.03, 95% CI 1.71–5.36; *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 with 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 tables S3 and 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 figures S1 and S2. CLAD-free and graft survival analysis applying different cut-offs for *early versus* late AFOP (*i.e.* 3 m, 6 m, 9 m, 12 m, 18 m, 24 m) is provided in figures 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.* [6] 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. 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 histopathological analysis of explant lungs, which revealed the presence of AFOP in approximately 50% of clinically identified RAS patients [7]. In contrast, Paraskeva *et al.* [6] did not observe histological changes consistent with AFOP in patients with a "RAS-like" clinical phenotype (but without TLC confirmation) [6].

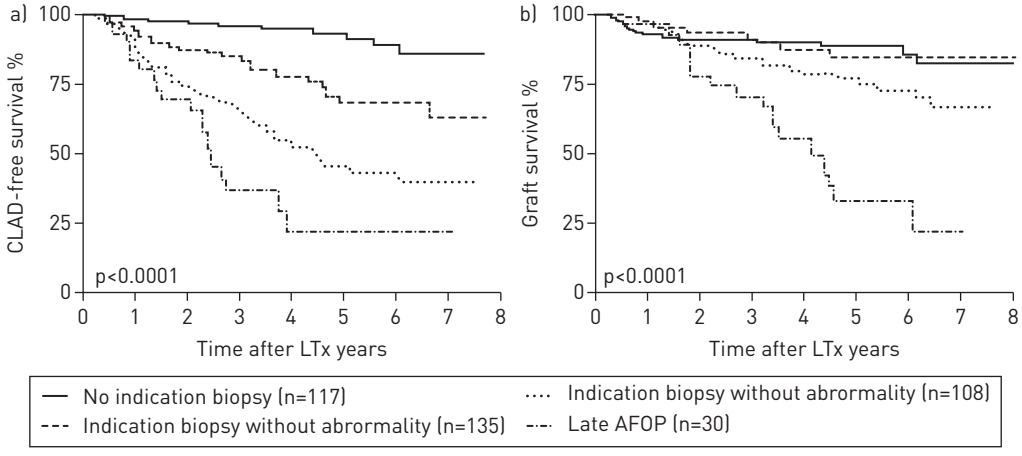

FIGURE 4 a) Kaplan–Meier curve illustrating chronic lung allograft dysfunction (CLAD)-free survival post-lung transplantation (LTx). b) Kaplan–Meier curve illustrating graft survival post-LTx. CLAD-free survival and graft survival are significantly worse in patients with late AFOP compared with patients with an indication biopsy with abnormality, patients with an indication biopsy without abnormality or patients without indication biopsy (p<0.0001 and p<0.0001, respectively). Patients with a graft survival of ⩽90 days post-LTx (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.

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 histopathological 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 histopathological patterns (*i.e.* late new-onset DAD and organising 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 histological patterns (*e.g.* DAD, eosinophilic pneumonia) may resemble the histopathological 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 $\geqslant$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. Secondly, this study is based on data from single-centre patients and, although we report a large patient cohort, extrapolation of these results to draw general conclusions about lung transplant patients might be restricted. Thirdly, 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 with 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 histopathological feature in the early pathogenesis of RAS. Further characterisation 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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