Successful *Pseudomonas aeruginosa* eradication improves outcomes after lung transplantation: a retrospective cohort analysis

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Presence of *P. aeruginosa* in respiratory samples after lung transplantation is associated with worse outcomes. Successful eradication improves outcomes and pulmonary function. Therefore, early treatment of *P. aeruginosa* should be pursued. https://bit.ly/2XuDPG2

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ABSTRACT Long-term survival after lung transplantation (LTx) is hampered by development of chronic lung allograft dysfunction (CLAD). *Pseudomonas aeruginosa* is an established risk factor for CLAD. Therefore, we investigated the effect of *P. aeruginosa* eradication on CLAD-free and graft survival.

Patients who underwent first LTx between July, 1991, and February, 2016, and were free from CLAD, were retrospectively classified according to *P. aeruginosa* presence in respiratory samples between September, 2011, and September, 2016. *P. aeruginosa*-positive patients were subsequently stratified according to success of *P. aeruginosa* eradication following targeted antibiotic treatment. CLAD-free and graft survival were compared between *P. aeruginosa*-positive and *P. aeruginosa*-negative patients; and between patients with or without successful *P. aeruginosa* eradication. In addition, pulmonary function was assessed during the first year following *P. aeruginosa* isolation in both groups.

CLAD-free survival of *P. aeruginosa*-negative patients (n=443) was longer compared with *P. aeruginosa*-positive patients (n=95) (p=0.045). Graft survival of *P. aeruginosa*-negative patients (n=443, 82%) was better compared with *P. aeruginosa*-positive patients (n=95, 18%) (p<0.0001). Similarly, *P. aeruginosa*-eradicated patients demonstrated longer CLAD-free and graft survival compared with patients with persistent *P. aeruginosa*. Pulmonary function was higher in successfully *P. aeruginosa*-eradicated patients compared with unsuccessfully eradicated patients (p=0.035).

P. aeruginosa eradication after LTx improves CLAD-free and graft survival and maintains pulmonary function. Therefore, early *P. aeruginosa* detection and eradication should be pursued.

Introduction

Lung transplantation (LTx) is an accepted treatment possibility in selected patients with end-stage lung diseases. However, long-term survival after LTx is limited, with a 5-year survival of approximately 60% worldwide, mainly due to development of chronic lung allograft dysfunction (CLAD) [1]. Bronchiolitis obliterans syndrome (BOS) is the main phenotype of CLAD and is histologically characterised by progressive loss of bronchial epithelium, chronic neutrophilic inflammation and fibroproliferation causing small airway obliteration or obliterative bronchiolitis [2–5].

Since the lung is in direct contact with the external environment, it is uniquely susceptible to microbial invasion. Therefore, infection and colonisation with viruses, bacteria and fungi of the allograft are highly prevalent. Moreover, colonisation and infection with these micro-organisms have previously been associated with BOS development [6–10]. In particular, *Pseudomonas aeruginosa*, a gram-negative aerobic rod [11], is one of the most common pathogens present after transplantation [12, 13]. Multiple studies demonstrated an association between *P. aeruginosa* and BOS [11, 14–17].

The pathogenesis of chronic *P. aeruginosa* colonisation and infection on the one hand, and BOS on the other hand, has not been fully clarified. However, chronic *P. aeruginosa* colonisation or infection leads to persistent inflammation, thereby causing epithelium damage [18, 19], which may lead to release of pro-inflammatory cytokines and epithelial alarmins such as interleukin (IL)-1α, followed by stimulation of the immune system and activation of pro-inflammatory fibroblasts [16, 20].

A similar evolution from chronic colonisation/infection to end-stage respiratory disease is seen in cystic fibrosis. Multiple retrospective and a systematic review performed, showed a benefit of aggressive *P. aeruginosa* treatment in cystic fibrosis patients, by avoiding evolution from transient to chronic infection and consequently preventing pulmonary function decline [21].

Given the similar detrimental effects of *P. aeruginosa* on airway inflammation and pulmonary function in LTx recipients, we hypothesise that *P. aeruginosa* eradication may lead to a decrease of CLAD development in LTx recipients; and hence in increased graft survival.

Material and methods

Study design

Single lung, bilateral lung and heart-lung transplant recipients, transplanted between July, 1991, and February, 2016, at the University Hospitals Leuven, were included in this retrospective single-centre study (figure 1). Patients without sufficient pulmonary function data, who underwent retransplantation or developed CLAD before September, 2011, (since graft and CLAD-free survival were assessed from September, 2011, onwards) were excluded. In all remaining LTx patients, all respiratory samples collected between September, 2011, and September, 2016, were evaluated for the presence of *P. aeruginosa*. As of September, 2011, we adopted an approach to prospectively eradicate *P. aeruginosa* once isolated from a respiratory sample by using susceptibility-directed targeted antibiotic treatment in all our LTx recipients in follow-up, given the risk of *P. aeruginosa* on later CLAD development. Patients with respiratory samples positive for *P. aeruginosa*, obtained after discharge following LTx, were subsequently categorised according to successful eradication or not. End of follow-up was February, 2018 (follow-up of at least 2 years). Endpoints included CLAD-free survival and graft survival. In addition, eradication treatment regimens were assessed in both groups. This study was approved by our local Ethics Committee and all patients gave written informed consent to access their clinical electronic medical records and biobanking data for research (S51577/ML5629).

Definitions

Definitions concerning eradication were based on the Cochrane Review by Hewer and Smyth [22]. In brief, attempt to eradication was the clinical decision of starting oral (*p.o.*) and/or intravenous (*i.v.*) antibiotics following *P. aeruginosa* isolation from a respiratory sample, in order to achieve successful eradication. Successful eradication was defined as absence of *P. aeruginosa* in all respiratory samples collected within 6 months following specific eradication treatment or no treatment (spontaneous eradication). Unsuccessful eradication was defined as isolation of *P. aeruginosa* in at least one of the

The data that support the findings of this study are available on request from the corresponding author.

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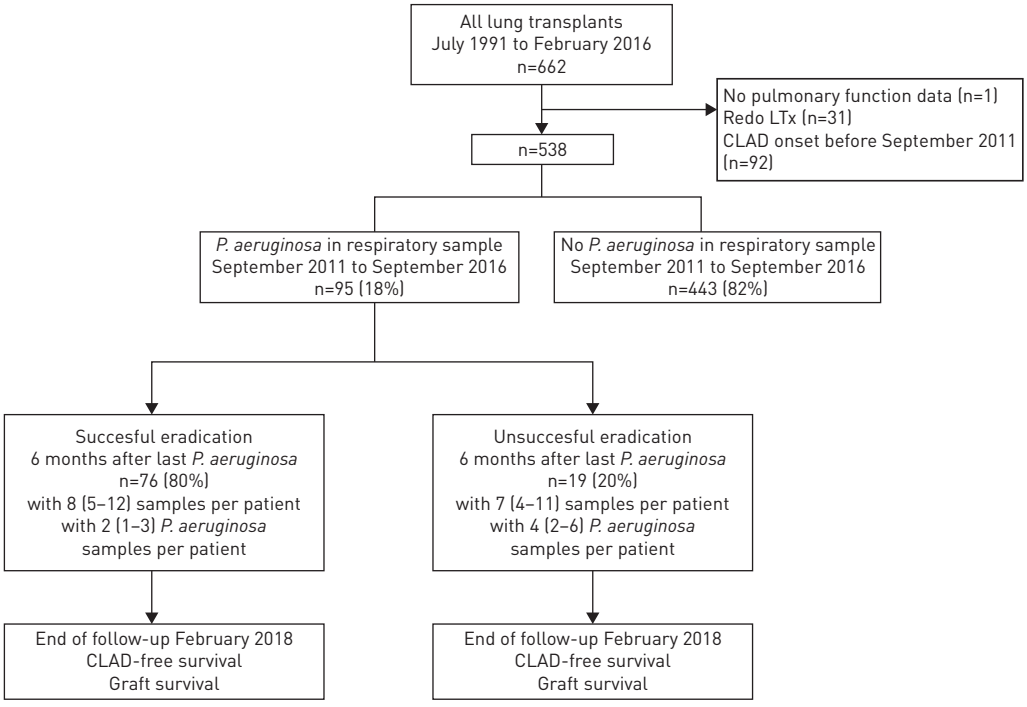

FIGURE 1 Flowchart diagram of study cohort. Patients transplanted between July 1991, and February 2016, (n=662) were screened, where after patients without data (n=1), who underwent retransplantation (n=31) or developed chronic lung allograft dysfunction (CLAD) before September, 2011, (start of study period) (n=92) were excluded. The remaining patients were screened for *Pseudomonas aeruginosa* presence in respiratory samples after discharge post-lung transplantation (LTx) between September, 2011, and September, 2016. Patients with positive *P. aeruginosa* respiratory samples were subsequently categorised according to successful (n=76, 80%) or unsuccessful eradication (n=19, 20%). At the end of follow-up (February 2018), both groups were assessed for CLAD-free survival, graft survival and eradication regimen.

respiratory samples collected within 6 months following specific (or no) eradication treatment. Patients without 6 months of follow-up after *P. aeruginosa* isolation (*e.g.* retransplantation or death), were considered successfully eradicated for analyses when the last available respiratory sample was negative for *P. aeruginosa*, and considered unsuccessfully eradicated when the last available respiratory sample was positive for *P. aeruginosa*. Multidrug-resistant *P. aeruginosa* was defined as *P. aeruginosa* showing acquired non-susceptibility to at least one agent in three or more antimicrobial categories [23].

CLAD was defined as a persistent decline in forced expiratory volume in 1 s (FEV_1) of ⩾20% from baseline (the mean of the best two post-operative FEV_1 measurements taken >3 weeks apart) which could not be explained by other conditions [24]. Pulmonary function tests were performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines [25]. CLAD-free survival was defined as the time from September, 2011 (at which moment all included patients were free from CLAD), until CLAD development, whereas graft survival included the time from September, 2011, until retransplantation or death (figure 2).

Respiratory samples

Respiratory samples were defined as sputum samples or bronchoalveolar lavage (BAL) samples, collected during routine follow-up, or in case of clinical respiratory symptoms or deteriorating pulmonary function. Routine transplant monitoring was previously described and is summarised in in the supplementary material [26]. Bronchoscopy with BAL was performed with two 50-mL aliquots of sterile saline in a subsegmental bronchus of the right middle lobe, or lobe of interest demonstrating radiographic abnormalities [26]. BAL fluid was recovered after each instillation by gentle manual suction. Fractions were pooled for microbiological and virological assessment. For microbiological and virological evaluation, 10 µL of sputum sample or 100 µL of BAL fluid was cultured into five different media (blood, mannitol salt, MacConkey, Haemophilus-selective and Sabouraud agar). Other media were used depending on clinical suspicion. The presence of one or more bacterial colonies after 48 h of incubation and fungal colonies after 3 weeks of incubation was considered significant and was reported in a standardised, semi-quantitative manner (−, +, ++, +++). Additionally, antibiotic susceptibility testing was performed

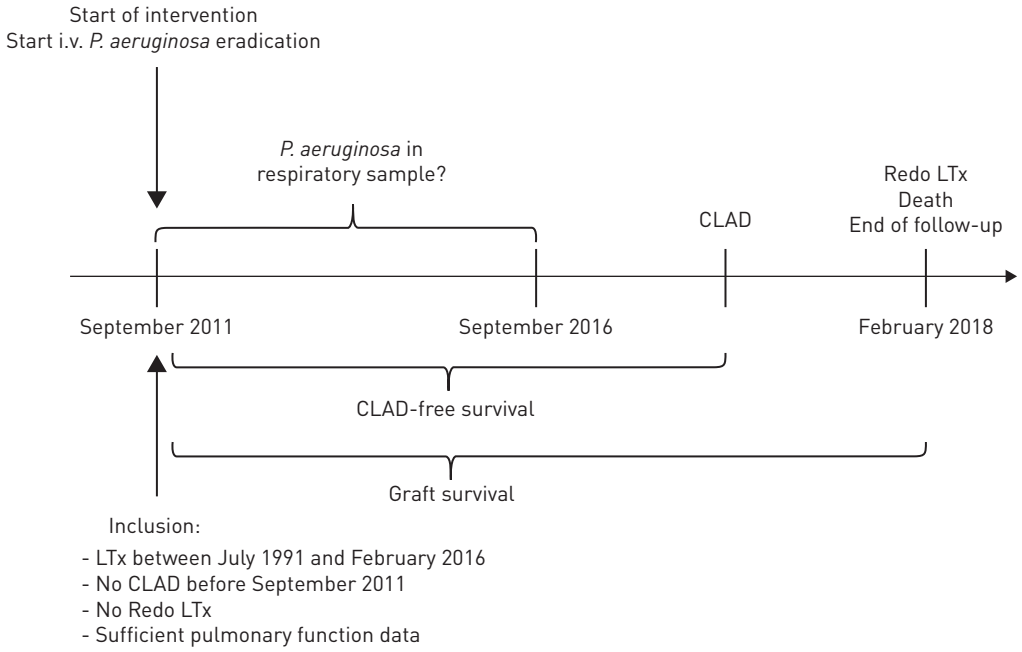

FIGURE 2 Timeline of study. Lung transplantation (LTx) recipients transplanted between July, 1991, and February, 2016, and who met inclusion criteria, were reviewed for *Pseudomonas aeruginosa* presence in respiratory samples after discharge post-LTx between September, 2011 and September, 2016. Patients with *P. aeruginosa*-positive respiratory samples were categorised according to successful eradication or not. Chronic lung allograft dysfunction (CLAD)-free survival and graft survival were compared between both groups. CLAD-free survival was considered as time from inclusion until CLAD development or from September, 2011, until CLAD development when patients were transplanted before the study period. Graft survival was defined as time from inclusion until retransplantation or death or from September, 2011, until retransplantation or death when patients were transplanted before the start of the study period.

according to standard microbiological protocols for amikacin, amoxicillin, cefepime, ceftazidime, sulfamethoxazole, levofloxacin, meropenem, piperacillin/tazobactam, tobramycin, vancomycin, ceftazidime/avibactam.

Transplant monitoring and therapeutic management

Routine transplant monitoring, postoperative prophylactic treatment and immunosuppressive regimen were previously described; and are summarised in the supplementary material [26]. In addition, our centre does not use standard sinus surgery after LTx, which is only performed in selected cases, based upon the patients' complaints, evolution on computed tomography of the sinuses and at the treating physician's discretion [27].

Statistical analysis

The primary endpoints of this study, including CLAD-free survival and graft survival, were analysed with the Kaplan–Meier method using the log-rank testing. The secondary endpoint, FEV_1 at first *P. aeruginosa* isolation, 6 and 12 months later, was, because of missing values, analysed using a linear mixed-effect model after $\log_{10}$ transformation of data. The model included time, group and time×group interaction.

Patient characteristics were summarised with descriptive statistics. Patients' proportions were compared using a Chi-squared test. Continuous data are shown as mean with standard error of mean when normally distributed; and as median with interquartile range when not normally distributed. Unpaired two-tailed t-tests and Mann–Whitney U-tests were used to compare group means in case of normally and non-normally distributed variables, respectively.

Statistical analysis was performed with GraphPad Prism 8.1.2 software (San Diego, CA, USA). A p-value of <0.05 was considered statistically significant.

Results

Study population

In our centre, 662 patients underwent single, bilateral or heart–lung transplantation between July, 1991, and February, 2016, and were still alive in September, 2011. Patients without sufficient data (n=1), who underwent retransplantation (n=31) or developed CLAD before September, 2011 (n=92) were excluded,

resulting in a population of 538 (81%) LTx recipients. In 18% of these patients (n=95), *P. aeruginosa* was isolated out of at least one respiratory sample between September, 2011, and September, 2016, whereas in 82% of remaining patients (n=443), no *P. aeruginosa* was isolated (figure 1).

P. aeruginosa versus *no* P. aeruginosa

LTx recipients with at least one *P. aeruginosa*-positive respiratory sample (n=95) were mostly transplanted for emphysema (43%), cystic fibrosis (41%), interstitial lung disease (ILD) (12%) or for pulmonary hypertension (4%). In contrast, LTx recipients without *P. aeruginosa*-positive respiratory samples (n=443) were mostly transplanted for emphysema (60%), ILD (19%), cystic fibrosis (14%), pulmonary hypertension (6%) or for another reason (1%) (p<0.0001). *P. aeruginosa*-positive patients were transplanted younger, at a median age of 46 years (29–59), compared to *P. aeruginosa*-negative patients, who were transplanted at a median age of 55 years (45–60) (p=0.0003). At the end of follow-up, more *P. aeruginosa*-negative patients were alive (82%), in comparison with *P. aeruginosa*-positive patients (65%) (p=0.0016). There were no significant differences in type of transplantation or sex (table 1).

CLAD-free survival at end of follow-up was significantly better in *P. aeruginosa*-negative patients compared with *P. aeruginosa*-positive patients, demonstrating a 5-year CLAD-free survival of 63% *versus* 54% (figure 3a). Similarly, graft survival at the end of follow-up was significantly better in *P. aeruginosa*-negative patients compared with *P. aeruginosa*-positive patients, demonstrating a 5-year graft survival of 98% *versus* 70% respectively (p=0.045) (figure 3b).

Successful eradication versus *unsuccessful eradication*

In 76 out of 95 (80%) *P. aeruginosa*-positive patients, eradication treatment was successful, whereas in 19 patients (20%) eradication treatment was unsuccessful (figure 1).

Patients successfully eradicated for *P. aeruginosa* had fewer *P. aeruginosa*-positive samples (2 (1–3) samples *versus* 4 (2–6) samples; p=0.0005) and more *P. aeruginosa*-negative samples (6 (3–10) samples *versus* 3 (1–7) samples; p=0.04) compared with unsuccessfully *P. aeruginosa*-eradicated patients. There was no significant difference in total number of respiratory samples between both groups (p=0.45). At the end of follow-up, more successfully eradicated patients (n=58, 76%) were still alive, compared with unsuccessfully eradicated patients (n=4, 21%) (p<0.001). Unsuccessfully *P. aeruginosa*-eradicated patients were more frequently donor-specific antibody-positive compared with successfully *P. aeruginosa*-eradicated patients (26% *versus* 11%; p=0.048). There were no significant differences in other patient characteristics (table 2).

TABLE 1 Patient characteristics of *Pseudomonas aeruginosa versus* no *Pseudomonas aeruginosa* group

	Pseudomonas aeruginosa	**No *Pseudomonas aeruginosa***	**p-value**
Patients	95 (18%)	443 (82%)	
Type of transplant			0.46
SSLTx	90 (95%)	403 (91%)	
SLTX	4 (4%)	29 (7%)	
HLTx	1 (1%)	11 (2%)	
Underlying disease			**<0.0001**
Emphysema	41 (43%)	266 (60%)	
Cystic fibrosis	39 (41%)	63 (14%)	
Interstitial lung disease	11 (12%)	82 (19%)	
Pulmonary hypertension	4 (4%)	27 (6%)	
Others	0 (0%)	5 (1%)	
Sex male	53 (56%)	214 (48%)	0.19
Age at transplantation years	46 (29–59)	55 (45–60)	**0.0003**
Outcome (February, 2018)			**0.0016**
Alive	62 (65%)	362 (82%)	
Redo LTx	5 (5%)	10 (2%)	
Death	28 (29%)	71 (16%)	
LTx era year	2011 (2007–2013)	2011 (2008–2013)	0.43

Data are presented as n (%) or median (interquartile range), unless otherwise stated. SSLTx: sequential single lung transplantation; SLTx: single lung transplantation; HLTx: heart-lung transplantation; LTx: lung transplantation.

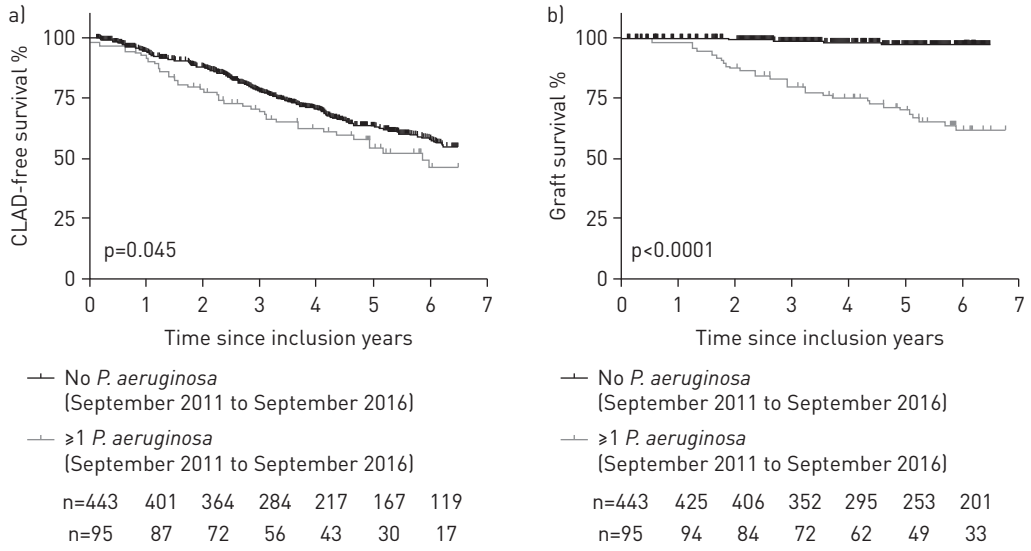

FIGURE 3 Graft survival and chronic lung allograft dysfunction (CLAD)-free survival of *Pseudomonas aeruginosa versus* no *P. aeruginosa*. a) Kaplan–Meier estimates of CLAD-free survival of patients without *P. aeruginosa*-positive respiratory samples (n=443, 82%) *versus* at least one *P. aeruginosa*-positive respiratory sample during study period (n=95, 18%) (p=0.045). b) Kaplan–Meier estimates of graft survival of patients without *P. aeruginosa*-positive respiratory samples (n=443, 82%) *versus* at least one *P. aeruginosa*-positive respiratory sample during study period (n=95, 18%) (p<0.0001). Time point 0 is time of inclusion of every patient during the study period (time of LTx) or September 1, 2011, when patients were transplanted before. A p-value of <0.05 was considered statistically significant.

Overall, successfully *P. aeruginosa*-eradicated patients demonstrated better CLAD-free and graft survival compared to unsuccessfully *P. aeruginosa*-eradicated patients during follow-up (figure 4a–h, supplementary legend).

In unsuccessfully *P. aeruginosa*-eradicated LTx recipients, more multidrug resistance *P. aeruginosa* was detected, compared to successfully *P. aeruginosa*-eradicated patients (58% *versus* 20%; p=0.0008). Both successfully and unsuccessfully *P. aeruginosa*-eradicated patients were treated mainly with *i.v.* antibiotics. Both groups were treated mainly with a combination of *i.v.* antibiotics (supplementary table S2). Duration of antibiotic treatment was similar in both groups (14 (10–21) days *versus* 14 (10–17) days; p=0.90) (supplementary table S2).

Pulmonary function

At the time of first *P. aeruginosa* isolation between September, 2011, and September, 2016, successfully *P. aeruginosa*-eradicated patients had a similar FEV_1 compared with unsuccessfully *P. aeruginosa*-eradicated patients (2.39 L±0.10 *versus* 2.23 L±0.22; p=0.48). However, successfully *P. aeruginosa*-eradicated patients had a better FEV_1 during the first year following first *P. aeruginosa* isolation, compared with unsuccessfully *P. aeruginosa*-eradicated patients (time×group interaction p=0.035). Successfully *P. aeruginosa*-eradicated patients had a stable pulmonary function during the first year, with a FEV_1 of 2.35±0.10 L at 6 months after first *P. aeruginosa* isolation and an FEV_1 of 2.35±0.11 L at 12 months after first *P. aeruginosa* isolation. On the other hand, unsuccessfully *P. aeruginosa*-eradicated patients demonstrated a decline in pulmonary function with an FEV_1 of 2.03±0.30 L at 6 months after first *P. aeruginosa* isolation and an FEV_1 of 1.89±0.31 L at 12 months after first *P. aeruginosa* isolation (figure 5).

Discussion

In this retrospective study of prospectively treated LTx recipient with *P. aeruginosa*, we demonstrated, for the first time, a significantly better graft survival and CLAD-free survival in patients with successful *P. aeruginosa* eradication. We also confirmed that presence of *P. aeruginosa* in respiratory samples is associated with CLAD development and decreased graft survival [11, 14–17].

In addition, pulmonary function was better in the first year following first *P. aeruginosa* isolation in successfully *P. aeruginosa*-eradicated patients compared with unsuccessfully *P. aeruginosa*-eradicated patients. These results are comparable to previous findings in cystic fibrosis patients, in whom stable pulmonary function has been demonstrated after successful *P. aeruginosa* eradication and decreasing pulmonary function is seen in case of chronic *P. aeruginosa* infection [28]. Our results show that early

TABLE 2 Patient characteristics of successful eradication *versus* unsuccessful eradication

	Successful eradication	Unsuccessful eradication	p-value
Patients	76 (80%)	19 (20%)	
Respiratory samples per patient n	8 (5–12)	7 (4–11)	0.45
Pseudomonas aeruginosa-positive samples per patient	2 (1–3)	4 (2–6)	**0.0005**
Pseudomonas aeruginosa-negative samples per patient	6 (3–10)	3 (1–7)	**0.04**
***Pseudomonas aeruginosa* presence before LTx**	48 (63%)	7 (37%)	>0.99
Samples per patient			**0.006**
1	38 (50%)	3 (16%)	
2–4	31 (41%)	10 (53%)	
5–9	7 (9%)	6 (32%)	
Type of transplant			0.86
SSLTx	72 (95%)	18 (95%)	
SLTX	3 (4%)	1 (5%)	
HLTx	1 (1%)	0 (0%)	
Underlying disease			0.47
Emphysema	30 (39%)	11 (58%)	
Cystic fibrosis	34 (45%)	5 (26%)	
Interstitial lung disease	9 (12%)	2 (11%)	
Pulmonary hypertension	3 (4%)	1 (5%)	
Sex male	41 (54%)	12 (63%)	0.47
Age at transplantation years	44 (28–59)	54 (37–61)	0.22
Outcome (February 2018)			**<0.0001**
Alive	58 (76%)	4 (21%)	
Redo LTx	14 (18%)	1 (5%)	
Death	4 (5%)	14 (74%)	
Era of LTx year	2011 (2008–2013)	2011 (2001–2014)	0.58
DSA			**0.048**
Positive	8 (11%)	5 (26%)	
Negative	42 (55%)	5 (26%)	
Unknown	26 (34%)	9 (47%)	

Data are presented as n (%) or median (range), unless otherwise stated. SSLTx: sequential single lung transplantation; SLTx: single lung transplantation; HLTx: heart-lung transplantation; LTx: lung transplantation; DSA: donor specific antibodies.

detection and eradication of *P. aeruginosa* should be pursued, similarly to the policy in cystic fibrosis patients [22].

The choice for a specific treatment regimen was a clinical decision made by the treating physician, directed by the actual antibiotic susceptibility of the identified *Pseudomonas* spp. strains. Therefore, associations with type of treatment (*i.v.*, *i.v.+p.o.*, *p.o.*) and success of eradication cannot be made, given the large heterogeneity of therapies used and absence of a standardised treatment protocol. However, this could be interesting for further research.

In our study, there was a significant difference in underlying disease in patients with or without *P. aeruginosa*-positive respiratory samples. In the *P. aeruginosa*-positive respiratory group, there were more cystic fibrosis patients compared with the *P. aeruginosa*-negative group. These results confirm the already-published findings that cystic fibrosis patients are more prone to *P. aeruginosa* colonisation, probably due to the adaptation of the bacteria to a mucoid phenotype (surviving in a biofilm) and due to re-infection of the lung allograft from the reservoir of the sinuses [27, 29]. In addition, *P. aeruginosa*-positive patients were, in general, younger compared with *P. aeruginosa*-negative patients, probably because there are more cystic fibrosis patients in the first group [1].

Importantly, outcome was better in *P. aeruginosa*-negative patients and successfully *P. aeruginosa*-eradicated patients, demonstrating for the first time that modification of an established risk factor for CLAD (presence of *P. aeruginosa* in respiratory samples), can affect relevant clinical long-term outcomes.

Inflammation and fibrosis are both important characteristics of BOS, though the underlying pathogenesis has not been fully clarified. In addition, the role of infections and colonisation, in particular with

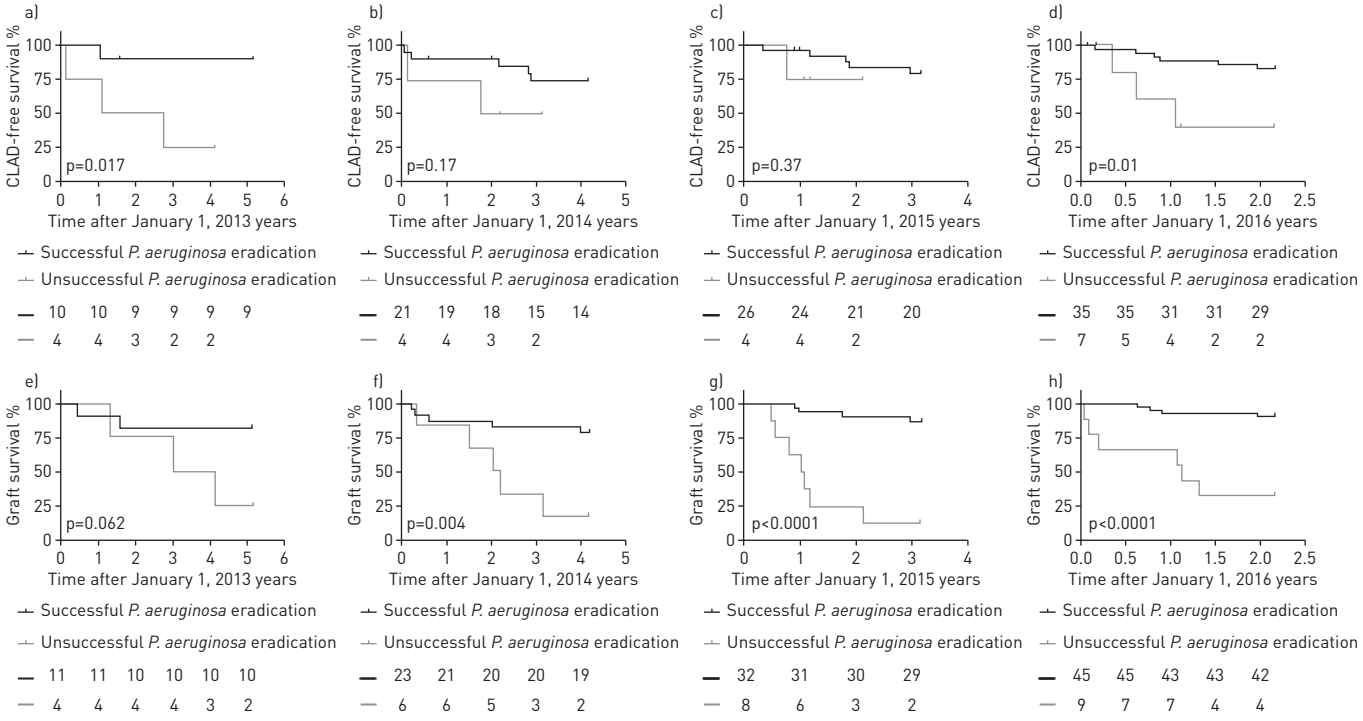

FIGURE 4 Graft survival and chronic lung allograft dysfunction (CLAD)-free survival of successful *Pseudomonas aeruginosa* eradication *versus* unsuccessful *Pseudomonas aeruginosa* eradication. Kaplan–Meier estimates of CLAD-free survival of patients with successful *Pseudomonas aeruginosa* eradication *versus* unsuccessful *Pseudomonas aeruginosa* eradication starting from time point (a) January 1, 2013, (b) January 1, 2014, (c) January 1, 2015 and (d) January 1, 2016. Only patients who were already transplanted, were alive and had not yet been diagnosed with CLAD, were included. Kaplan–Meier estimates of graft survival of patients with successful *Pseudomonas aeruginosa* eradication *versus* unsuccessful *Pseudomonas aeruginosa* eradication starting from time point (e) January 1, 2013, (f) January 1, 2014, (g) January 1, 2015 and (h) January 1, 2016. Only patients who were already transplanted and were alive, were included.

P. aeruginosa, is not well understood. However, accumulating evidence shows that chronic *P. aeruginosa* colonisation/infection causes epithelial damage [18, 19]. Subsequently, this may lead to release of pro-inflammatory cytokines and epithelial alarmins (such as IL-1α), followed by immune activation, epithelial-to-mesenchymal transition and fibroproliferation [16, 20]. We hypothesise that early *P. aeruginosa* detection and eradication may prevent ongoing epithelial damage and, in this way, may

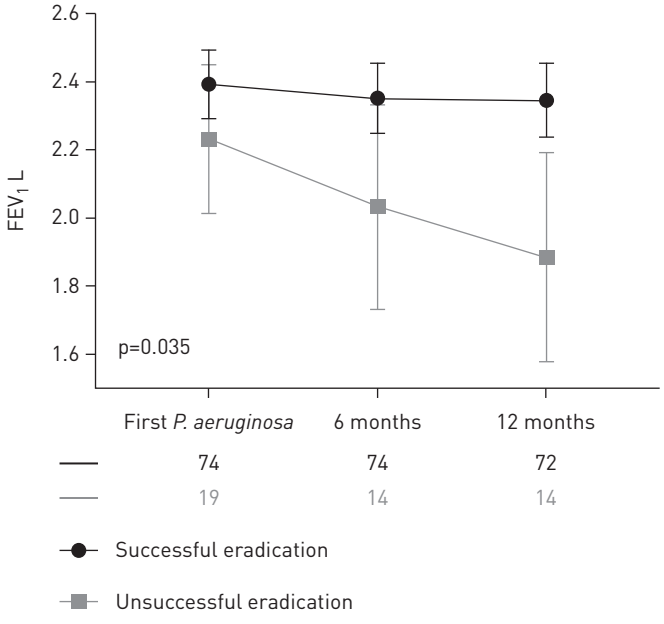

FIGURE 5 Pulmonary function in successfully *P. aeruginosa*-eradicated patients *versus* unsuccessfully *P. aeruginosa*-eradicated patients. Successfully *P. aeruginosa*-eradicated patients demonstrate better forced expiratory volume in 1 s (FEV_1) in the first year following first *P. aeruginosa* isolation (September, 2011, to September, 2016) compared with unsuccessfully eradicated patients. FEV_1 was analysed using a linear mixed effect model after log10 transformation of data. The model included time, group and time×group interaction effect. A p-value of <0.05 was considered statistically significant.

prevent the deleterious vicious circle of pro-inflammatory cytokine and epithelial alarmin release, chronic inflammation with stimulation of the immune system and activation of fibroblasts. More specifically, Borthwick *et al.* [16] showed that IL-1α was elevated in LTx recipients with chronic *P. aeruginosa* infection. IL-1α can induce IL-8 secretion by bronchial epithelial cells, which leads to increased airway neutrophilia [30]. In addition, previous work of our group showed that also IL-1β may play a central role in LTx recipients with high airway neutrophilia despite azithromycin treatment, which were characterised by more prevalent *P. aeruginosa* colonisation and worse CLAD-free survival compared with azithromycin treated LTx recipients with low BAL-neutrophilia [31]. Taken together, these findings pave the way for further mechanistic and therapeutic studies with a central role of IL-1, especially in *P. aeruginosa*-positive patients. In addition, it is also possible that *P. aeruginosa* only thrives in damaged airways, and could be considered a marker rather than a driver for CLAD. Unfortunately, this exceeds the scope of our clinical study.

Moreover, successfully eradicated *P. aeruginosa* patients were significantly less DSA positive compared with unsuccessfully *P. aeruginosa*-eradicated patients. This result supports recently published evidence, were a significant association between *P. aeruginosa* isolation (and other inflammatory events like episodes of A-grade and B-grade rejection) and development of DSAs to mismatched DQ alleles has been shown [32]. These findings suggest that innate immune responses in turn can activate humoral alloimmunity after LTx

Evidently, our study has limitations. First, this was a retrospective study; however, all our LTx recipients receive life-long 3–4 monthly follow-up in our centre, allowing standardised follow-up. BAL samples and sputum samples were collected on fixed time points and in case of clinical respiratory symptoms or respiratory function decline. Moreover, bronchoscopic procedure was performed and respiratory samples were processed in a standardised way, as previously described. Nevertheless, because of the retrospective design of our study and the limitation of available data, we were unable to investigate some important questions. For example, it will be interesting in the future to prospectively study the effect of *P. aeruginosa* colonisation before LTx and the duration of *P. aeruginosa* colonisation before *P. aeruginosa*-eradication treatment on the success rate of *P. aeruginosa* eradication. In addition, because of the study design, cohorts were retrospectively assigned, which can confound our analyses. Also, accepted definitions of eradication/colonisation in LTx recipients are lacking. Therefore, we based our definitions on the definitions used in cystic fibrosis [22], in which extensive experience is available. In addition, patient numbers were rather limited, a limitation inherent to all single-centre studies in LTx recipients. Therefore, multi-centre studies are needed. Lastly, our study aimed to answer some clinically important questions, leaving underlying mechanisms out of its scope. As a consequence, we were only able to hypothesise about, but not unravel, any new underlying pathogenic mechanisms.

In conclusion, eradication of *P. aeruginosa* in lung transplant recipients is associated with improved graft survival, CLAD-free survival and pulmonary function. Therefore, early detection and eradication of *P. aeruginosa* should be pursued.

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