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Early View

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

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SUCCESSFUL *PSEUDOMONAS AERUGINOSA* ERADICATION IMPROVES OUTCOMES AFTER LUNG TRANSPLANTATION: A RETROSPECTIVE COHORT ANALYSIS

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ABSTRACT

Background

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

Methods

Patients who underwent first LTx between 07/1991 - 02/2016 and were free from CLAD, were retrospectively classified according to *PA* presence in respiratory samples between 09/2011 and 09/2016. *PA* positive patients were subsequently stratified according to success of *PA* eradication following targeted antibiotic treatment. CLAD-free and graft survival were compared between *PA* positive and *PA* negative patients; and between patients with or without successful *PA* eradication. In addition, pulmonary function was assessed during the first year following *PA* isolation in both groups.

Results

CLAD-free survival of *PA* negative patients (n=443) was longer compared to *PA* positive patients (n=95) (p=0.045). Graft survival of *PA* negative patients (n=443, 82%) was better compared to *PA* positive patients (n=95, 18%) (p<0.0001). Similarly, *PA* eradicated patients demonstrated longer CLAD-free survival compared to patients with persistent *PA* (p=0.018). Pulmonary function was higher in successfully *PA* eradicated patients compared to unsuccessfully eradicated patients (p=0.035).

Conclusion

PA eradication after LTx improves CLAD-free and graft survival and maintains pulmonary function. Therefore, early *PA* 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 characterized 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 colonization with viruses, bacteria and fungi of the allograft are highly prevalent. Moreover, colonization and infection with these micro-organisms have previously been associated with BOS development (6–10). In particular, *Pseudomonas aeruginosa* (*PA)*, a gram-negative aerobic rod (11), is one of the most common pathogens present after transplantation (12,13). Multiple studies demonstrated an association between *PA* and BOS (11,14–17).

The pathogenesis of chronic *PA* colonization and infection on the one hand, and BOS on the other hand, has not been fully clarified. However, chronic *PA* colonization 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 IL-1α, followed by stimulation of the immune system and activation of pro-inflammatory fibroblasts (16,20).

A similar evolution from chronic colonization/infection to end-stage respiratory disease is seen in cystic fibrosis (CF). Multiple retrospective and a systematic review performed, showed a benefit of aggressive *PA* treatment in CF patients, by avoiding

evolution from transient to chronic infection and consequently preventing pulmonary function decline (21).

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

MATERIAL & METHODS

Study design

Single lung, bilateral lung and heart-lung transplant recipients, transplanted between 07/1991 and 02/2016 at the University Hospitals Leuven, were included in this retrospective single center study (figure 1). Patients without sufficient pulmonary function data, who underwent retransplantation or developed CLAD before 09/2011 (since graft and CLAD-free survival were assessed from 09/2011 on) were excluded. In all remaining LTx patients, all respiratory samples collected between 09/2011 and 09/2016 were evaluated for the presence of *PA*. As of 09/2011, we adopted an approach to prospectively eradicate *PA* 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 *PA* on later CLAD development. Patients with respiratory samples positive for *PA*, obtained after discharge following LTx, were subsequently categorized according to successful eradication or not. End of follow-up was 02/2018 (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 a Langton Hewer and Smith Cochrane Review (22). In brief, attempt to eradication was the clinical decision of starting oral (PO) and/or intravenous (IV) antibiotics following *PA* isolation from a respiratory sample, in order to achieve successful eradication. Successful eradication was defined as absence of *PA* in all respiratory samples collected within 6 months following specific eradication treatment (or no treatment, spontaneous eradication). Unsuccessful eradication

was defined as isolation of *PA* in at least one of the respiratory samples collected within 6 months following specific (or no) eradication treatment. Patients without 6 months of follow-up after *PA* isolation (e.g. retransplantation or death), were considered successfully eradicated for analyses when the last available respiratory sample was negative for *PA*, and considered unsuccessfully eradicated when the last available respiratory sample was positive for *PA*. Multidrug resistance *PA* was defined as *PA* showing acquired non-susceptibility to at least one agent in 3 or more antimicrobial categories (23).

CLAD was defined as a persistent decline in forced expiratory volume in one second (FEV_1) of $\geq$20% from baseline (the mean of the best 2 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 ATS/ERS guidelines (25). CLAD-free survival was defined as the time from 09/2011 (at which moment all included patients were free from CLAD) until CLAD development, whereas graft survival included the time from 09/2011 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 summarized in in the supplementary data (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 hours of incubation and fungal colonies after 3 weeks of incubation was considered significant and was reported in a standardized, semi-quantitative manner (-, +, ++, +++). Additionally, antibiotic susceptibility testing was performed 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 summarized in the supplementary data (26). In addition, our center does not use standard sinus surgery after LTx, which is only performed in selected cases, based upon the patients' complaints, evolution on CT 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 analyzed with the Kaplan-Meier method using the log-rank testing. The secondary endpoint, FEV_1 at first *PA* isolation, 6 and 12 months later, was, because of missing values, analyzed using a linear mixed effect model after $\log_{10}$ transformation of data. The model included time, group and time*group interaction.

Patient characteristics were summarized with descriptive statistics. Patients' proportions were compared using Chi-square 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). P-values of <0.05 were considered statistically significant.

RESULTS

Study population

In our center, 662 patients underwent single, bilateral or heart-lung transplantation between 07/1991 - 02/2016 and were still alive 09/2011. Patients without sufficient data (n=1), who underwent retransplantation (n=31) or developed CLAD before 09/2011 (n=92) were excluded, resulting in a population of 538 (81%) LTx recipients. In 18% of these patients (n=95), *PA* was isolated out of at least one respiratory sample between 09/2011 - 09/2016, whereas in 82% of remaining patients (n=443), no *PA* was isolated (figure 1).

Pseudomonas aeruginosa versus no Pseudomonas aeruginosa

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

CLAD-free survival at end of follow-up was significantly better in *PA* negative patients compared to *PA* 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 *PA* negative patients compared to *PA* 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 of 95 (80%) *PA* positive patients, eradication treatment was successful, whereas in 19 patients (20%) eradication treatment was unsuccessful (figure 1).

Patients successfully eradicated for *PA* had less *PA* positive samples (2 (1-3) samples versus 4 (2-6) samples, p=0.0005) and more *PA* negative samples (6 (3-10) samples versus 3 (1-7) samples, p=0.04) compared to unsuccessfully *PA* 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 to unsuccessfully eradicated patients (n=4, 21%) (p<0.001). Unsuccessfully *PA* eradicated patients were more frequently DSAs positive compared to successfully *PA* eradicated patients (26% versus 11%, p=0.048). There were no significant differences in other patient characteristics (table 2).

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

In unsuccessfully *PA* eradicated LTx recipients, more multidrug resistance *PA* was detected, compared to successfully *PA* eradicated patients (20% versus 58%, p=0.0008). Both successfully and unsuccessfully *PA* eradicated patients were treated mainly with IV antibiotics. Both groups were treated mainly with a combination of IV antibiotics (*Supplementary table 2)*. Duration of antibiotic treatment was similar in both groups (14 (10-17) days versus 14 (10-21) days, p=0.90) (*Supplementary table 2*).

Pulmonary function

At the time of first *PA* isolation between 09/2011 - 09/2016, successfully *PA* eradicated patients had a similar FEV_1 compared to unsuccessfully *PA* eradicated patients (2.39L±0.10 versus 2.23L±0.22, p=0.48). However, successfully *PA* eradicated patients had a better FEV_1 during the first year following first *PA* isolation, compared to unsuccessfully *PA*

eradicated patients (time*group interaction p=0.035). Successfully *PA* eradicated patients had a stable pulmonary function during the first year, with a FEV_1 of 2.35±0.10L at 6 months after first *PA* isolation and a FEV_1 of 2.35±0.11L at 12 months after first *PA* isolation. On the other hand, unsuccessfully *PA* eradicated patients demonstrated a decline in pulmonary function with a FEV_1 of 2.03±0.30L at 6 months after first *PA* isolation and a FEV_1 of 1.89±0.31L at 12 months after first *PA* isolation.

DISCUSSION

In this retrospective study of prospectively treated LTx recipient with *PA*, we demonstrated for the first time, a significantly better graft survival and CLAD-free survival in patients with successful *PA* eradication. We also confirmed that presence of *PA* 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 *PA* isolation in successfully *PA* eradicated patients compared to unsuccessfully *PA* eradicated patients. These results are comparable to previous findings in CF patients, in which stable pulmonary function has been demonstrated after successful *PA* eradication and decreasing pulmonary function is seen in case of chronic *PA* infection (28). Our results show that early detection and eradication of *PA* should be pursued, similarly to the policy in CF 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* strains. Therefore, associations with type of treatment (IV, IV+PO, PO) and success of eradication cannot be made, given the large heterogeneity of therapies used and absence of a standardized 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 *PA* positive respiratory samples. In the *PA* positive respiratory group, there were more CF patients compared to the *PA* negative group. These results confirm the already published findings that CF patients are more prone for *PA* colonization, 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, *PA* positive patients were in general younger compared to *PA* negative patients, probably because there are more CF patients in the first group (1).

Importantly, outcome was better in *PA* negative patients and successfully *PA* eradicated patients, demonstrating for the first time that modification of an established risk factor for CLAD (i.e., presence of *PA* 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 colonization, in particular with *PA*, is not well understood. However, accumulating evidence shows that chronic *PA* colonization/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 hypothesize that early *PA* detection and eradication may prevent ongoing epithelial damage and, in this way, may 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 and colleagues showed that IL-1α was elevated in LTx recipients with chronic *PA* infection (16). 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 characterized by more prevalent *PA* colonization and worse CLAD-free survival compared to 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 *PA* positive patients. Unfortunately, this exceeds the scope of our clinical study. In addition, it is also possible that PA 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 *PA* patients were significantly less DSA positive compared to unsuccessfully *PA* eradicated patients. This result supports recently published evidence, were a significant association between *PA* 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 response 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 center, allowing standardized 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 standardized way, as previously described. Nevertheless, because of the respective 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 *PA* colonization before LTx and the duration of *PA* colonization before *PA* eradication treatment on the success rate of *PA* eradication. In addition, because of the study design, cohorts were retrospectively assigned, which can confound our analyses. Also, accepted definitions of eradication/colonization in LTx recipients are lacking. Therefore, we based our definition on the definition used in CF (22), in which extensive experience is available. In addition, patient numbers were rather limited, a limitation inherent to all single-center studies in LTx recipients. Therefore, multi-center 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 hypothesize about, but not unravel, any new underlying pathogenic mechanisms.

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

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Disclosures:

None of the authors of this manuscript has any conflicts of interest to disclose in relation with this manuscript. The authors confirm that that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form in English or in any other language, without the written consent of the copyright holder.

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TABLES

Tabel 1. Patient characteristics of *Pseudomonas aeruginosa* versus no *Pseudomonas aeruginosa* group

	PA	**No *PA***	**p-value**
Patients, n (%)	95 (18%)	443 (82%)	
Type of transplant, n (%)			0.46
SSLTx	90 (95%)	403 (91%)	
SLTX	4 (4%)	29 (7%)	
HLTx	1 (1%)	11 (2%)	
Underlying Disease, n (%)			**<0.0001**
Emphysema	41 (43%)	266 (60%)	
Cystic Fibrosis	39 (41%)	63 (14%)	
ILD	11 (12%)	82 (19%)	
PH	4 (4%)	27 (6%)	
Others	0 (0%)	5 (1%)	
Gender (males), n (%)	53 (56%)	214 (48%)	0.19
Age at transplantation, yr	46 (29-59)	55 (45-60)	**0.0003**
Outcome (02/2018), n (%)			**0.0016**
Alive	62 (65%)	362 (82%)	
Redo LTx	5 (5%)	10 (2%)	
Death	28 (29%)	71 (16%)	
LTx era, yr	2011 (2007-2013)	2011 (2008-2013)	0.43

Abbreviations: *PA: Pseudomonas aeruginosa*; SSLTx: sequential single lung transplantation; SLTx: single lung transplantation; HLTx: heart-lung transplantation; ILD: interstitial lung disease; PH: pulmonary hypertension; LTx: lung transplantation; yr(s): year(s).

Table 2. Patient characteristics of successful eradication versus unsuccessful eradication

	Successful eradication	Unsuccessful eradication	p-value
Patients, n (%)	76 (80%)	19 (20%)	
Respiratory samples per patient, n	8 (5-12)	7 (4-11)	0.45
PA pos samples per patient, n	2 (1-3)	4 (2-6)	**0.0005**
PA neg samples per patient, n	6 (3-10)	3 (1-7)	**0.04**
PA presence before LTx, n(%)	48 (63%)	7 (37%)	>0.99
Samples per patient, n(%)			**0.006**
1	38 (50%)	3 (16%)	
2-4	31 (41%)	10 (53%)	
5-9	7 (9%)	6 (32%)	
Type of transplant, n (%)			0.86
SSLTx	72 (95%)	18 (95%)	
SLTX	3 (4%)	1 (5%)	
HLTx	1 (1%)	0 (0%)	
Underlying Disease, n (%)			0.47
Emphysema	30 (39%)	11 (58%)	
Cystic Fibrosis	34 (45%)	5 (26%)	
ILD	9 (12%)	2 (11%)	
PH	3 (4%)	1 (5%)	
Gender (males), n (%)	41 (54%)	12 (63%)	0.47
Age at transplantation, yr	44 (28-59)	54 (37-61)	0.22
Outcome (02/2018), n (%)			**<0.0001**
Alive	58 (76%)	4 (21%)	
Redo LTx	14 (18%)	1 (5%)	
Death	4 (5%)	14 (74%)	
Era of LTx, yr	2011 (2008-2013)	2011 (2001-2014)	0.58
DSA, n(%)			**0.048**
Positive	8 (11%)	5 (26%)	
Negatieve	42 (55%)	5 (26%)	
Unknown	26 (34%)	9 (47%)	

Abbreviations: *PA: Pseudomonas aeruginosa*; SSLTx: sequential single lung transplantation; SLTx: single lung transplantation; HLTx: heart-lung transplantation; ILD: interstitial lung disease; PH: pulmonary hypertension; LTx: lung transplantation; yr(s): year(s), DSA: donor specific antibodies.

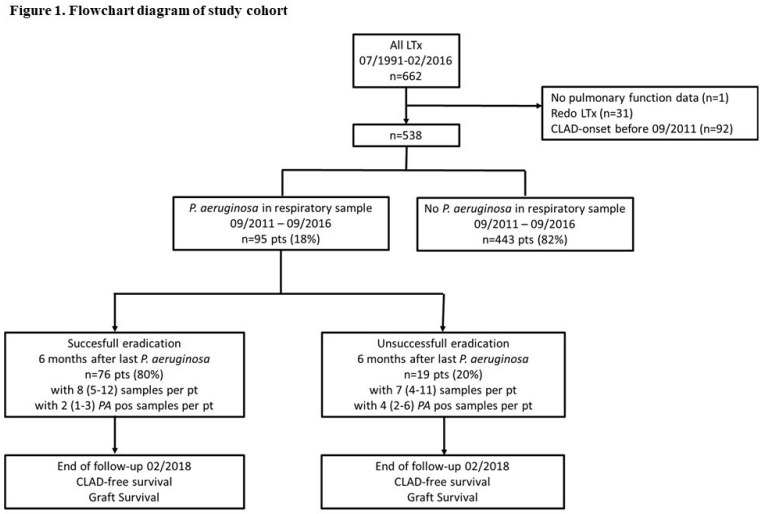


Figure 1. Flowchart diagram of study cohort

Patients transplanted between 07/1991 and 02/2016 (n=662) were screened, where after patients without data (n=1), who underwent retransplantation (n=31) or developed CLAD before 09/2011 (start of study period) (n=92) were excluded. The remaining patients were screened for PA presence in respiratory samples after discharge post-LTx between 09/2011 - 09/2016. Patients with positive PA respiratory samples were subsequently categorized according to successful (n=76, 80%) or unsuccessful eradication (n=19, 20%). At the end of follow-up (02/2018), both groups were assessed for CLAD-free survival, graft survival and eradication regimen.

Abbreviations: LTx: lung transplantation; CLAD: chronic lung allograft dysfunction; PA: Pseudomonas aeruginosa.

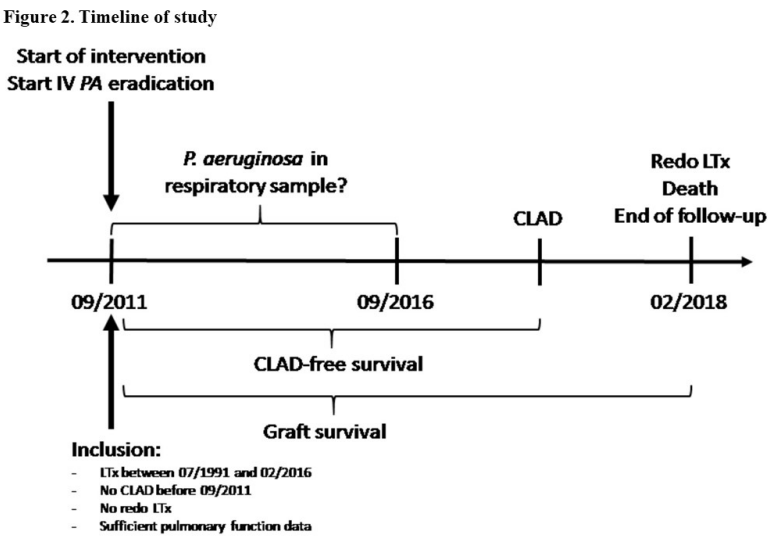


Figure 2. Timeline of study

LTx recipients transplanted between 07/1991 - 02/2016 and who met inclusion criteria, were reviewed for PA presence in respiratory samples after discharge post-LTx between 09/2011 - 09/2016. Patients with PA positive respiratory samples were categorized according to successful eradication or not. 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 09/2011 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 09/2011 until retransplantation or death when patients were transplanted before the start of the study period.

Abbreviations: PA: Pseudomonas aeruginosa; CLAD: chronic lung allograft dysfunction; LTx: lung transplantation.

Figure 3. Graft survival and CLAD-free survival of *Pseudomonas aeruginosa* versus no *Pseudomonas aeruginosa*

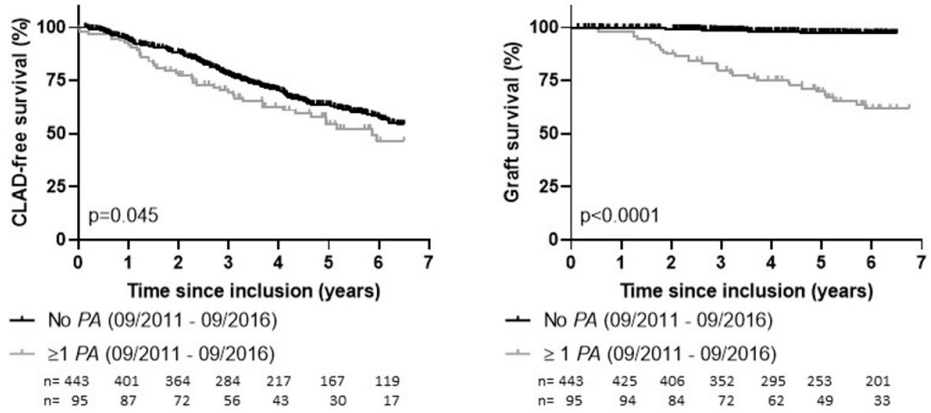


Figure 3. Graft survival and CLAD-free survival of Pseudomonas aeruginosa versus no Pseudomonas aeruginosa

A) Kaplan-Meier estimates of CLAD-free survival of patients without PA positive respiratory samples (n=443, 82%) versus at least 1 PA positive respiratory sample during study period (n=95, 18%) (p=0.045). B) Kaplan-Meier estimates of CLAD-free survival of patients without PA positive respiratory samples versus at least 1 PA 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 01/09/2011 when patients were transplanted before. A p-value of < 0.05 was considered statistically significant.

Abbreviations: PA: Pseudomonas aeruginosa; CLAD: chronic lung allograft dysfunction.

Figure 4. Graft survival and CLAD-free survival of successful *PA* eradication versus unsuccessful *PA* eradication

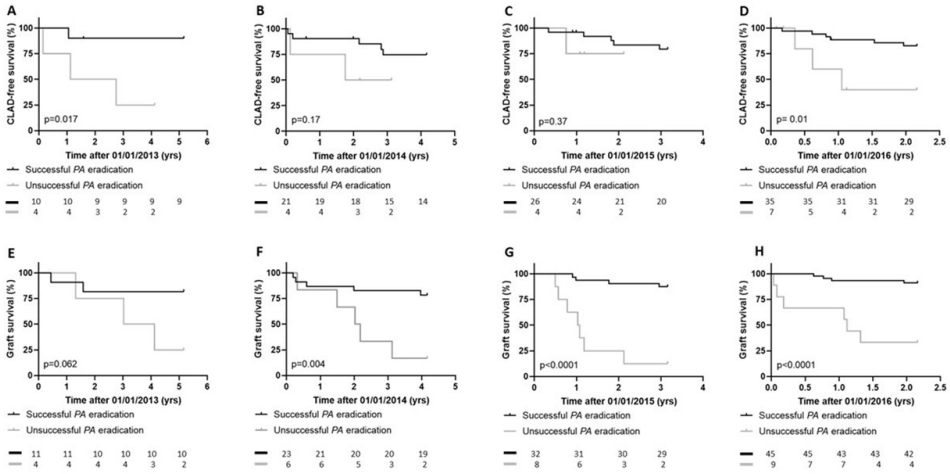
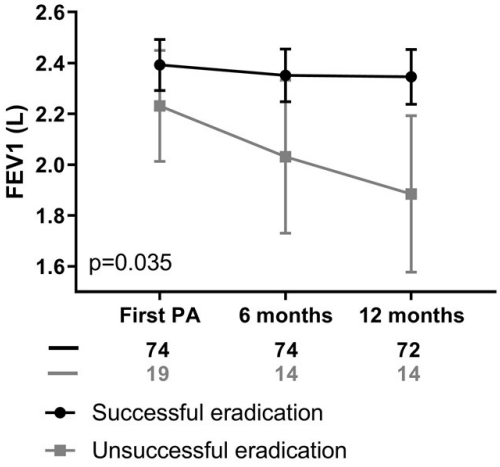


Figure 4. Graft survival and CLAD-free survival of successful PA eradication versus unsuccessful PA eradication

Kaplan-Meier estimates of CLAD-free survival of patients with successful PA eradication versus unsuccessful PA eradication starting from time point (A) 01/01/2013, (B) 01/01/2014, (C) 01/01/2015 and (D) 01/01/2016. Only patients who were already transplanted, were alive or who had not yet been diagnosed with CLAD, were included. Kaplan-Meier estimates of graft survival of patients with successful PA eradication versus unsuccessful PA eradication starting from time point (E) 01/01/2013, (F) 01/01/2014, (G) 01/01/2015 and (H) 01/01/2016. Only patients who were already transplanted and were alive, were included.

Abbreviations: PA: Pseudomonas aeruginosa; CLAD: chronic lung allograft dysfunction.

Figure 5. Pulmonary function in successfully *PA* eradicated patients versus unsuccessfully *PA* eradicated patients



Successfully PA eradicated patients demonstrate better FEV1 in the first year following first PA isolation (09/2011-09/2016) compared to unsuccessfully eradicated patients. FEV1 was analyzed 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.

Abbreviations: PA: Pseudomonas aeruginosa; FEV1: forced expiratory volume in one second

ONLINE SUPPLEMENTARY DATA

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Transplant monitoring

After discharge, LTx recipients received routine follow-up at fixed time points: twice a week during the first 2-4 weeks after discharge, then once weekly until 12 weeks after LTx, every 4 weeks until 6 months after LTx, every 6-8 weeks until 12 months after LTx, and thereafter life-long at intervals of 3-4 months. In addition, patients performed home spirometry and were instructed to come to the outpatient clinic in case of fever or >10% FEV_1 decline. During each patient contact, complete history and physical examination was performed as well as blood, urine, sputum and pharyngeal swab cultures, spirometry and chest radiography. In addition, chest CT and bronchoscopic evaluation with broncho-alveolar lavage (BAL), transbronchial biopsies (TBB) and/or endobronchial biopsies were performed at discharge and 3, 6, 12, 18 and 24 months after LTx, and whenever clinically indicated. When a FEV_1 decline of ≥ 20% was noticed, chest CT and bronchoscopy with BAL and TBB were performed to exclude acute causes for pulmonary function decline.

Therapeutic management

Following induction therapy with anti-thymocyte globulin (rATG) (3 mg/kg/d for 3 days), patients received conventional triple-drug immunosuppressive maintenance therapy consisting of methylprednisolone, a cytostatic agent (azathioprine or mycophenolate mofetil) and a calcineurin inhibitor (cyclosporine A or tacrolimus). Drug choice was made according to the discretion of the treating clinician, dose adjustments were made based on renal function and immunosuppressive trough levels. Acute rejection (grade A2 or higher) was treated by high dose corticosteroids during 3 days, tapered to oral maintenance dose over the next 2 to 3 weeks. Grade A1 acute rejection was treated by augmenting oral steroids, similarly followed by tapering. Isolated grade B rejection was treated with azithromycin (250 mg/day, trice weekly) and corticosteroids in case of ≥B2R similar to the protocol of acute rejection.

In suspected CLAD, immunosuppressive treatment was optimized if possible and azithromycin (250 mg/day, trice weekly) was initiated. In established, progressive CLAD, treatment with montelukast (MLK) 10 mg/day was started. In case of subsequent CLAD progression (i.e. further decline in FEV_1 after ≥ 3-6 months of MLK), rescue-treatment with pulsed steroids, rATG, TLI, pirfenidone, antibody-directed therapy (pulsed methylprednisolone, followed by plasmapheresis, intravenous immunoglobulins (IVIG) and rituximab since 2013), or retransplantation was performed in selected cases, based on the treating physician's discretion. Extracorporeal photopheresis (ECP) is not commonly available in our center for CLAD.

In addition, all LTx recipients were routinely treated with a low dose proton pomp inhibitor. If reflux was diagnosed after LTx by either pH impedance measurement or gastroscopy, low dose proton pump inhibitor was switched to high dose proton pump inhibitor. None of the included patients underwent fundoplication surgery after LTx.

After LTx, conventional infectious prophylaxis for cytomegalovirus, Aspergillus spp., and Pneumocystis spp was started. CMV-related disease or pneumonitis was treated with intravenous ganciclovir (5 mg/kg twice daily for at least two weeks or lower based on eGFR); Aspergillus infection was treated with voriconazole (4 mg per kg bid IV). Antibiotic treatment for bacterial infection after LTx was guided using bacteriologic cultures.

Supplementary tables

Table1. Number of samples in successfully and unsuccessfully PA eradicated patients

	Successful eradication	**Unsuccessful eradication**	**p-value**
Respiratory samples per patient, n	8 (5-12)	7 (4-11)	0.45
PA pos respiratory samples, n	2 (1-3)	4 (2-6)	**0.0005**
PA neg respiratory samples, n	6 (3-10)	3 (1-7)	**0.04**
Sputum samples per patient, n	3 (1-7)	3 (2-5)	0.96
PA pos sputum samples, n	1 (0-2)	2 (0-4)	**0.03**
PA neg sputum samples, n	2 (0-6)	1 (0-3)	0.30
BAL samples per patient, n	5 (2-6)	3 (1-5)	0.96
PA pos BAL samples, n	1 (0-2)	1 (0-2)	0.18
PA neg BAL samples, n	4 (1-5)	0 (1-4)	**0.02**

Table 1. Number of positive and negative respiratory (sputum and BAL), sputum and BAL samples in successfully and unsuccessfully *PA* eradicated patients.

Table 2. Eradication regimen

	Successful eradication	**Unsuccessful eradication**	**p-value**
Patients, n (%)	76 (80%)	19 (20%)	
Eradication Treatment, n (%)	53	13	**0.02**
IV antibiotics	31 (58%)	13 (100%)	
IV and PO antibiotics	7 (13%)	0 (0%)	
PO antibiotics	15 (28%)	0 (0%)	
No eradication treatment	23	6	
IV antibiotics, n (%)			
Ceftazidime	8 (21%)	4 (31%)	0.48
Colistin	6 (16%)	5 (38%)	0.09
Meropenem	18 (47%)	9 (69%)	0.17
Piperacillin/tazobactam	19 (50%)	3 (23%)	0.09
Tobramycin	16 (42%)	4 (31%)	0.47
Combination treatment	25 (66%)	10 (77%)	0.46
Days of IV antibiotics, days	14 (10-17)	14 (10-21)	0.90
PO antibiotics, n (%)			
Levofloxacin	12 (82%)	0 (0%)	NA
Ciprofloxacin	8 (36%)	0 (0%)	NA
Moxifloxacin	2 (9%)	0 (0%)	NA
Days of PO antibiotics, days	14 (9-14)	NA	NA

Table 2. Eradication treatment in successfully versus unsuccessfully *PA* eradicated patients. Inhaled antibiotics are not routinely used because they are not reimbursed after LTx in Belgium.

Supplementary legend figure 4

At 01/01/2013, 11 of the included LTx patients were successfully *PA* eradicated (of which 1 was already diagnosed with CLAD), 4 were unsuccessfully *PA* eradicated (of which none were diagnosed with CLAD) and 80 were not transplanted, had no positive *PA* sample yet or already died. Successfully *PA* eradicated patients had a significantly better CLAD-free survival ($p=0.017$) and tended to have a better graft survival ($p=0.062$) compared to unsuccessfully *PA* eradicated patients (figure 4A and 4E).

At 01/01/2014, 23 of the included LTx patients were successfully *PA* eradicated (of which 2 were diagnosed with CLAD), 6 were unsuccessfully *PA* eradicated (of which 2 were diagnosed with CLAD) and 66 were not transplanted, had no positive *PA* sample yet or already died. Successfully *PA* eradicated patients tended to have a better CLAD-free survival ($p=0.17$, figure 4B) and had a better graft survival ($p=0.004$, figure 4F) compared to unsuccessfully *PA* eradicated patients.

At 01/01/2015, 32 of the included LTx patients were successfully *PA* eradicated (of which 6 were diagnosed with CLAD), 8 were unsuccessfully *PA* eradicated (of which 4 were diagnosed with CLAD) and 55 were not transplanted, had no positive *PA* sample yet or already died. Successfully *PA* eradicated patients had a significantly better graft survival compared to unsuccessfully *PA* eradicated patients ($p<0.0001$, figure 4G). There was no significant difference in CLAD-free survival between both groups ($p=0.37$, figure 4C).

At 01/01/2016, 45 of the included LTx patients were successfully *PA* eradicated (of which 10 were diagnosed with CLAD), 9 were unsuccessfully *PA* eradicated (of which 2 were diagnosed with CLAD) and 41 were not transplanted, had no positive *PA* sample yet or already died. Successfully *PA* eradicated patients had a significantly better CLAD-free ($p=0.01$, figure 4D) and graft survival ($p<0.0001$, figure 4H) compared to unsuccessfully *PA* eradicated patients ($p<0.0001$).