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Influence of mycophenolate mofetil dosage and plasma levels on the occurrence of chronic lung allograft dysfunction in lung transplants: a retrospective cohort analysis

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Summary

INTRODUCTION: Development of chronic lung allograft dysfunction is a limiting factor for post-lung transplant survival. We evaluated whether the dose of the immunosuppressant mycophenolate mofetil or plasma concentrations of the active metabolite mycophenolic acid affect the development of chronic lung allograft dysfunction.

METHODS: In this retrospective cohort study we recruited 71 patients with a lung transplant between 2010 and 2014 which survived the first year after transplantation up to 1 July 2021. An event-time-analytical Cox proportional-hazards regression model with time-varying-covariates (18,431 measurements for MPA, mycophenolate mofetil dosage, lymphocytes) was used to predict chronic lung allograft dysfunction, with adjustment for sociodemographic factors and lung function at baseline.

RESULTS: 37 patients did not develop chronic lung allograft dysfunction (age 41.3 ± 15.6 years, baseline FEV1 95.5 ± 19.1% predicted) and 34 patients developed chronic lung allograft dysfunction (age 50.9 ± 13.3 years, baseline FEV1 102.2 ± 25.4% predicted). Mean mycophenolic acid did not differ significantly between the groups (2.8 ± 1.7 and 3.0 ± 2.3 mg/l; p = 0.724). In the first 4 post-transplant years the death rate was 25%. A total of 50% of the patients died by the ninth post-transplant year. There was a dose-effect relationship between mycophenolate mofetil dosage, mycophenolic acid (r^2 = 0.02, p <0.001), as well as lymphocyte levels (r^2 = –0.007, p <0.001), but only the traditional risk factor age predicted chronic lung allograft dysfunction. Continuously measured mycophenolic acid did not predict chronic lung allograft dysfunction (hazard ratio 0.98, 95% confidence interval 0.90–1.06, p = 0.64 over a period of 382.97 patient-years).

CONCLUSION: Mycophenolate mofetil dosage and mycophenolic acid were not associated with chronic lung allograft dysfunction development. Thus, the mycophenolate mofetil dose or mycophenolic acid plasma concentration are not a primary factor related to organ rejection, but chronic lung allograft dysfunction may be influenced by other components of immunosuppression or other factors.

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Introduction

Lung transplantation is the ultimate therapy for patients with end-stage lung disease [1]. In 2017 alone, 4554 lung transplantation procedures were conducted worldwide, representing an increase of 50% within 10 years. Although improved peri- and intraoperative management, as well as optimised drug therapy, have positively influenced the post-transplantation survival rates, the mean survival after lung transplantation of 6.7 years remains lower than that associated with transplants of other solid organs [2–5].

The most common underlying diseases in patients who undergo lung transplantation are interstitial lung disease and a subgroup called idiopathic interstitial pneumonia, chronic obstructive pulmonary disease (COPD), and cystic fibrosis. Survival after transplantation varies depending on the underlying disease and also depends on the age of the recipient, with cystic fibrosis patients showing the longest survival (average 9.9 years) [2, 6].

An important limiting factor for survival is the development of chronic transplant rejection, the so-called chronic lung allograft dysfunction. The most common phenotype of chronic lung allograft dysfunction is bronchiolitis obliterans syndrome [2]. This has been identified the most common cause of mortality after lung transplantation over the past 30 years [7]. Chronic lung allograft dysfunction is diagnosed when patients show a persistent decrease of ≥20% in the forced expiratory volume within 1 s (FEV1) and other potentially reversible or irreversible causes have been ruled out [2, 8].

Precise and easy-to-use diagnostics to detect chronic lung allograft dysfunction at an early stage are crucial for post-transplant survival. In addition to lung function measure-

ments, imaging of the lungs and, to a lesser extent, laboratory analyses are used for recognising and classifying chronic lung allograft dysfunction. For example, chronic lung allograft dysfunction may present with increased levels of inflammatory parameters, lymphocytes and eosinophils in the blood [9]. Bronchoalveolar lavage (BAL) as a diagnostic tool for allograft rejection is currently being explored. The presence of neutrophilic or eosinophilic alveolitis in BAL fluid is considered a risk factor for chronic lung allograft dysfunction development. BAL is also an accepted method for ruling out pulmonary infections [10, 11]. Chronic lung allograft dysfunction was diagnosed when the patient showed an irreversible decrease of ≥20% in FEV1, and other causes such as heart failure, weight gain, lung infection or anastomotic complications were ruled out [2, 9].

At the University Hospital Zurich, lifelong triple therapy consisting of a corticosteroid, a calcineurin inhibitor (ciclosporin or tacrolimus), and an antimetabolite (often mycophenolate mofetil; Cellcept®) is used for immunosuppression after lung transplantation. Registry data showed that triple therapy with tacrolimus, mycophenolate mofetil and prednisone was administered to 62% of all lung transplant recipients between 2005 and 2018 [7]. Mycophenolate mofetil has largely replaced the previously used azathioprine, a purine analogue that also acts as an antimetabolite in immunosuppression after lung transplantation. Several studies have provided evidence for the use of mycophenolate mofetil by showing reduced incidence of lung allograft dysfunction, thus enabling longer survival [12, 13]. Therefore, mycophenolate mofetil is an important component of post-transplant immunosuppression.

The known variability in pharmacokinetics of other immunosuppressants, such as tacrolimus and ciclosporin, is believed to be less pronounced for mycophenolate mofetil. For this reason, mycophenolate mofetil is administered at a fixed dose and, unlike the drugs mentioned above, not regularly evaluated with therapeutic drug monitoring [1, 14]. After ingestion, mycophenolate mofetil is metabolised to the active metabolite mycophenolic acid, which inhibits the proliferation of T and B lymphocytes and thus the adaptive immune system [1, 12, 15]. mycophenolic acid is 97.5% protein-bound in plasma, with only the free form being pharmacologically active. The proportion of free mycophenolic acid may be increased in patients with reduced albumin levels or liver dysfunction. Mycophenolic acid is excreted as an inactive, protein-bound metabolite via the kidneys or in bile. However, in patients with kidney failure, this metabolite accumulates and displaces the mycophenolic acid from albumin, resulting in an increase of the free form [16]. Other factors that influence mycophenolic acid concentration include drug interactions, polymorphisms in enzymes involved in its breakdown, and the time after a transplant [16, 17]. However, over time, the pharmacokinetics of mycophenolic acid have been shown to vary more than previously thought. Most of the factors that increase this variability are associated with the metabolism or excretion of mycophenolate mofetil [16]. In addition, clinical data obtained after kidney transplantation showed a correlation between the incidence of transplant rejection and the measured plasma mycophenolic acid concentrations [18, 19]. For these reasons, in addition to evaluating the administered mycophenolate mofetil dose, the plasma mycophenolic acid concentration of the patients should be assessed and analysed in the context of the transplant function.

The main aim of this study was to analyse the trough mycophenolic acid concentrations (MPA_0) and mycophenolate mofetil dose in relation to the development of chronic lung allograft dysfunction. Mycophenolic acid concentration and mycophenolate mofetil dose were compared between patients with and without chronic lung allograft dysfunction.

Methods

Patients and data extraction

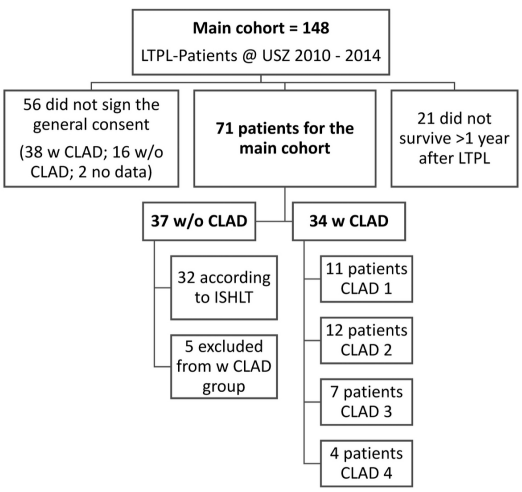
This was a retrospective cohort study. All patients who underwent bilateral lung transplantation between January 2010 and December 2014, and were older than 18 years were included in the analysis. Patients who did not survive the first post-transplant year or did not provide general consent for participation were excluded from the study population. Between January 2010 and December 2014, 148 patients underwent lung transplantation at the University Hospital Zurich. Twenty-one patients died within the first post-transplant year and were excluded because the survival time was too short for data analysis of chronic lung allograft dysfunction. In addition, 56 lung transplant recipients who did not provide general consent for participation in the study were not included in the study population. Of these 56 excluded patients, 38 had chronic lung allograft dysfunction and 16 showed no detectable chronic lung allograft dysfunction. Data were not available for 2 patients (fig. 1). Ultimately, a total of 71 patients who survived longer than 1 year and received triple immunosuppressive therapy consisting of a corticosteroid, mycophenolate mofetil and a calcineurin inhibitor (ciclosporin or tacrolimus) were evaluated in this study. Three of these patients were retransplanted at the time of the study entry and none of the patients received a retransplantation during the observation period. The study flow in figure 1 demonstrates the study population, who were divided into two groups: those who did not develop chronic lung allograft dysfunction (n = 37) and those who developed chronic lung allograft dysfunction (n = 34). A total of five patients were transferred from the group with chronic lung allograft dysfunction to the group without chronic lung allograft dysfunction due to heart failure, infection or weight gain (fig. 1). The observation period was extended from the date of transplantation to 1 July 2021. MPA_0 concentrations, mycophenolate mofetil doses and blood lymphocyte counts were recorded for patients with and without chronic lung allograft dysfunction on a daily basis from an individual day zero (day after transplantation) until an event (death or end of observation). The primary outcome was the association of mycophenolate mofetil dose and MPA_0 concentrations with the development of chronic lung allograft dysfunction. The secondary outcome was the association with overall survival. The lymphocyte levels were added as a confounder of interest. mycophenolate mofetil dose and MPA_0 were analysed from the day after the transplantation until the initial diagnosis of chronic

lung allograft dysfunction, patient death or the end of the observation period.

Definition of chronic lung allograft dysfunction

Chronic lung allograft dysfunction was defined as a persistent decrease of ≥20% in the forced expiratory volume within 1 s (FEV1), measured at least twice over at least 3 months, in comparison with the reference value (baseline). This reference value was calculated from the mean of the two best post-transplant FEV1 values within the first two post-transplant years [2]. Chronic lung allograft dysfunction was diagnosed when the patient showed a persistent drop of ≥20% in FEV1, and other potentially reversible or irreversible causes were ruled out [2, 8]. Chronic lung allograft dysfunction was staged (1–4) according to the current International Society for Heart and Lung Transplantation (ISHLT) recommendations and included both types of rejection, bronchiolitis obliterans syndrome and restrictive allograft syndrome, with bronchiolitis obliterans being the most common form of chronic lung allograft dysfunction (CLAD). CLAD stage 1 is characterised by current FEV1 >65% to 80% of the baseline value, CLAD stage 2 by FEV1 >50% to 65%, CLAD stage 3 by FEV1 >35% to 50%, CLAD stage 4 by FEV1 ≤35% [2]. Other typical causes that also reduced lung function and had to be ruled out prior to a diagnosis of chronic lung allograft dysfunction included heart failure, weight gain or lung infection. To identify heart failure, brain natriuretic peptide levels in the blood and echocardiography records were evaluated. To assess weight gain and lung infection, all weight data and C-reactive protein levels in the blood 3 months before and after the postulated chronic lung allograft dysfunction were analysed. The results were compared on the basis of the available lung histology data obtained from transbronchial biopsy, cell differentiation in BAL fluid, and thoracic computed tomography (CT) changes over a period of 6 months before and after the onset of chronic lung allograft dysfunction. The transbronchial biopsies were assessed by an experienced pulmonary pathologist, and chest CT scans were evaluated by an experienced chest radiologist to identify chronic lung allograft dysfunction changes.

Figure 1: Study flow. We detected 37 patients without and 34 patients with chronic lung allograft dysfunction. Five patients were transferred from the group with chronic lung allograft dysfunction to the group without chronic lung allograft dysfunction due to heart failure, infection, or weight gain. CLAD: chronic lung allograft dysfunction; ISHLT: International Society for Heart and Lung Transplantation; LTPL: lung transplantation; USZ: Universitätsspital Zürich.



Immunosuppression

All patients received triple immunosuppressive therapy, in which the corticosteroid dose after 1 year was 0.1 mg/kg/day rounded to 5 mg, 7.5 mg or 10 mg daily. The tacrolimus trough value to be achieved was 12–14 µg/l within the first 3 months, 9–12 µg/l after 3–9 months, and 6–9 µg/l after 9 months post-transplant. The target ciclosporin trough value was 250–300 µg/l in the first 3 months, which was adjusted further according to the area under the curve (AUC). Mycophenolate mofetil was initially administered at a dose of 3000 mg/day divided into two doses and then adjusted depending on the plasma mycophenolic acid trough concentration (MPA_0) as well as blood leucocyte and lymphocyte counts, with a target value >3000/µl for leucocytes and between 500 and 1500/µl for lymphocytes. The MPA_0 to be achieved was between 2 and 4 mg/, although this level was only measured as a secondary value indicative of drug resorption, since no therapeutic drug monitoring in the classic sense was carried out. Acute rejection was treated with steroid augmentation therapy and subsequent dose adjustments of ciclosporin or tacrolimus. In cases with mycophenolate mofetil doses <3000 mg/day, the mycophenolate mofetil doses were also increased, unless cytopenia prevented this. No generic immunosuppressive drugs were administered. The principles of post-lung-transplant immunosuppression in our patients were based on established drug target values that have been described previously [20, 21].

MPA0 measurements

Plasma mycophenolic acid trough concentration (MPA_0) was measured just before the next dose of mycophenolate mofetil in the morning. MPA_0 was analysed by means of an immunoassay, the so-called enzyme multiplied immunoassay technique (EMIT 2000 Mycophenolic Acid Assay, Siemens Healthcare Diagnostics) on the Konelab 30i measurement device (ThermoFisher Scientific) (measurement range 0.1–15 µg/ml; imprecision 14% at 1 µg/ml and 8% at 8 µg/ml). MPA_0 was usually measured repeatedly within 6 months after lung transplantation and every 6–12 months thereafter.

Statistical analysis

All statistical analyses were performed by the study team in consultation with statisticians. For baseline analyses, mean or median values were obtained according to the distribution of continuous variables. Proportions were determined for categorical variables. A t-test (for normally distributed continuous variables) and a Wilcoxon rank-sum test (for non-normally distributed continuous variables) were used for hypothesis testing. The $\chi 2$ test was used for categorical variables.

For the primary outcome (How does MPA_0 influence the development of chronic lung allograft dysfunction?), an event-time-analytical Cox proportional-hazards regression model (including log-rank test) was modelled with the

MPA_0 as the exposure factor (metric, time-varying covariate) and the chronic lung allograft dysfunction as a binary event (as defined in the introduction). This approach allowed for an interaction between time (on a daily level) and covariables (e.g., MPA_0) based on 18,431 different time intervals (each interval with a median time of 10 (IQR 2–24) days) clustered in 71 individuals respected the changes in influence on the outcome over time (table 2) [22]. Time zero for each patient was the day after transplantation. The following effect modifiers were subjected to a sensitivity analysis: time since transplantation, weight change since transplantation, presence of a pulmonary infection, and presence of heart failure. The results are presented as a hazard ratio (HR), and the reasons for censoring over the observation period were provided without exception (i.e., chronic lung allograft dysfunction, death or alive). For the secondary outcome a separate survival analysis was modelled. Regression analysis estimates were reported using 95% confidence intervals (CIs), and a two-tailed p-value of <0.05 was considered statistically significant for all tests reported. All statistical analyses were performed using STATA version 17.0 (StataCorp LP, College Station, TX). Approval from the Competent Ethics Committee was waived because of formal non-objection (BASEC-ID 2020– 2020–03024).

Results

Characterisation of the patients

A total of five patients were transferred from the group with chronic lung allograft dysfunction to the group without chronic lung allograft dysfunction due to heart failure, infection or weight gain. Finally, 34 and 37 patients were assigned to the groups with and without chronic lung allograft dysfunction, respectively. The group with chronic lung allograft dysfunction included 11 patients with CLAD stage 1, 12 with CLAD stage 2, 7 with CLAD stage 3, and 4 with CLAD stage 4 (fig. 1).

The mean age in the group without chronic lung allograft dysfunction was 41.3 ± 15.6 years, and that in the group with chronic lung allograft dysfunction was 50.9 ± 13.3 years; thus, patients with chronic lung allograft dysfunction were significantly older (p = 0.007). The group with chronic lung allograft dysfunction showed a balanced sex distribution (50% men), whereas the group without chronic lung allograft dysfunction showed a distribution slightly in favour of men (54% men). The underlying diseases are shown in table 1. The group without chronic lung allograft dysfunction showed a clear dominance of cystic fibrosis, followed by COPD. In contrast, COPD was the dominant underlying disease in patients with chronic lung allograft dysfunction. The baseline FEV1 did not differ significantly between patients without and with chronic lung allograft dysfunction (95.5 ± 19.1 and 102.2 ± 25.4; p = 205). Deaths before the diagnosis of chronic lung allograft dysfunction or before the end of the observation period occurred in 8 and 15 patients without and with chronic lung allograft dysfunction, respectively (table 1).

Association of groups and mycophenolate mofetil characteristics

The mean MPA_0 did not differ significantly between the groups without and with chronic lung allograft dysfunction (2.8 ± 1.7 mg/l and 3.0 ± 2.3 mg/l; p = 0.724). The same applied for average intra-individual MPA_0 standard deviation of MPA_0 (1.1 ± 0.8 mg/l and 1.1 ± 0.7 mg/l; p = 0.862). The average mycophenolate mofetil dosage in the groups without and with chronic lung allograft dysfunction also showed no statistically significant difference (1828 ± 533 and 1731 ± 676 mg; p = 0.511) as with the intra-individual standard deviation of average mycophenolate mofetil dosage (686 ± 211 mg/d and 661 ± 238 mg/d; p = 0.626).

Table 1:
Baseline characteristics of the main cohort.

Variable		Patients without CLAD (n = 37)	Patient with CLAD (n = 34)	p-value
Age, years		41.3 ± 15.68	50.90 ± 13.3	0.007
Male sex, n (%)		20 (54%)	17 (50%)	0.116
FEV1 (baseline), % predicted		95.5 ± 19.1	102.2 ± 25.4	0.205
Death before CLAD or censoring, n (%)		8 (34.8%)	15 (65.2%)	0.063
Causes of lung transplantation, n	A1AT	0	1	0.293
	Bronchiolitis	1	0	0.334
	CF	22	4	0.006
	COPD	7	16	0.011
	ILD	2	2	0.931
	LAM	0	3	0.065
	NA	0	1	0.293
	PH	1	1	0.952
	Pulmonary fibrosis	2	5	0.189
	Other	2	1	0.606
Calcineurin inhibitor, n (%)	Ciclosporin	28 (75.7%)	27 (79.4%)	0.370
	Tacrolimus	9 (24.3%)	7 (20.6%)	0.707
Comorbidities, n (%)	Heart failure	8 (21.6%)	4 (11.8%)	0.268
	Renal insufficiency	10 (27%)	9 (26.5%)	0.958

Values are displayed as n (%), FEV1 baseline % ± SD or median ± SD

CLAD: chronic lung allograft dysfunction; FEV1: forced expiratory volume within 1 s; A1AT: alpha-1-antitrypsin deficiency; CF: cystic fibrosis; COPD: chronic obstructive pulmonary disease; ILD: interstitital lung disease; LAM: lymphangioleiomyomatosis; PH: pulmonary hypertension

Association of lymphocytes with mycophenolate mofetil

A total of 1152 measurements of MPA_0 (median 9, IQR 5–15 per patient), 5653 data entries of mycophenolate mofetil dosage (median 45, IQR 21–79 per patient], and 15,812 measurements of lymphocytes (median 112, IQR 56–181 per patient) from all 71 patients were analysed. The lymphocyte counts showed a negative correlation with MPA_0 (r^2 = –0.007, p <0.001) and mycophenolate mofetil dosage (r^2 = –0.008, p <0.001), whereas MPA_0 and mycophenolate mofetil dosage were correlated positively (r^2 = 0.02, p <0.001).

Follow-up of the cohort

The observation period ended either with the development of a chronic lung allograft dysfunction (highest stage reached), the death of the patient, or the end of the observation period on 13 July 2021. Almost 50% of the patients developed chronic lung allograft dysfunction by the fifth post-transplant year (fig. 2). The Kaplan-Meier curve shows a death rate of 25% in the first 4 post-transplant years. A total of 50% of the patients had died by the ninth post-transplant year (fig. 3).

Cox regression

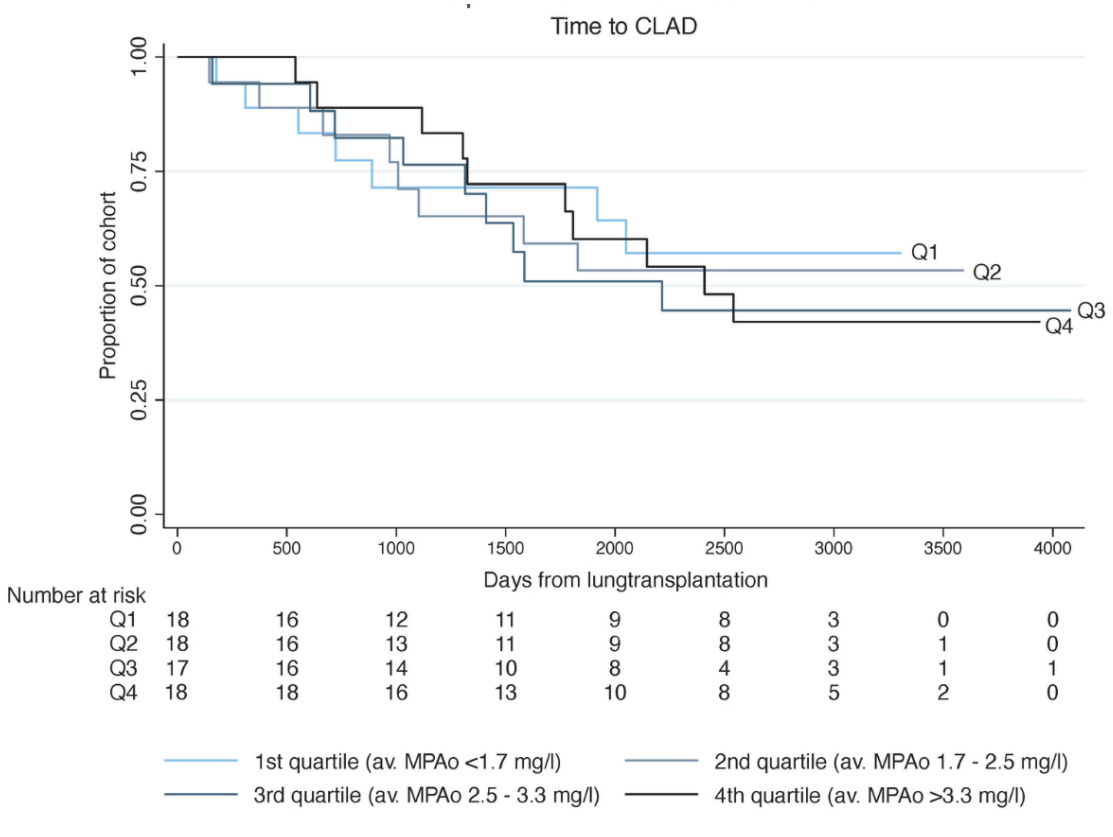
In a first model a univariable and multivariable event-time-analytical Cox regression model was made for chronic lung allograft dysfunction (table 2, fig. 2). A statistically significant association was observed between patient age and development of chronic lung allograft dysfunction. However, sex was not associated with chronic lung allograft dysfunction and the proportional hazards assumption for this variable was deemed valid (log-rank test for equality of survivor function: p = 0.750). The variables were analysed from the day after the lung transplant until the initial diagnosis of chronic lung allograft dysfunction (n = 34), patient death (n = 4), or the end of the observation period (n = 33). There was no association between MPA_0 and/or mycophenolate mofetil dosage with chronic lung allograft dysfunction over a period of 382.97 patient-years. In a post-hoc analysis, intra-individual MPA_0 variability was also not associated with the main outcome (HR 0.964, 95% CI 0.915–1.024; p = 0.795).

In a second model only survival data (22 deaths in 71 patients) were analysed (fig. 3) and there was no association between MPA_0 and the occurrence of death over an observation period of 472.8 patient-years (adjusted for the same covariables, HR 1.023, 95% CI 0.869–1.206; p = 0.782).

Radiological features of patients with chronic lung allograft dysfunction

At least one chest CT scan was performed in all 34 patients with chronic lung allograft dysfunction during the observation period (6 months before and after chronic lung allograft dysfunction diagnosis). Five of these patients did not show chest CT changes. Among the patients with chronic lung allograft dysfunction stages 1, 2, 3 and 4, 90.9%, 75%, 85.7%, and 100% showed changes indicating bronchiolitis obliterans in chest CT, including "air trapping" in the expiratory images, as well as fibrotic changes indicat-

Figure 2: Kaplan-Meier graph failure estimate Q. Almost 50% of the patients developed chronic lung allograft dysfunction by the fifth post-transplant year (i.e., after 1826 days). CLAD: chronic lung allograft dysfunction; MPA_0: trough concentration of mycophenolic acid.



ing restrictive allograft syndrome or mixed forms. In addition, 20% of transbronchial biopsies performed in cases with CLAD stage 1 and 30% of those performed in cases with CLAD stage 2 showed acute cellular rejection (table 3).

Figure 3: Kaplan-Meier graph survival estimate Q. In the first 4 post-transplant years (i.e., 1461 days) cumulative death-rate was 25%. A total of 50% of the patients died by the ninth post-transplant year (i.e., 3287 days). Patients who died within the first year were excluded from this study (conditional survival). CLAD: chronic lung allograft dysfunction; MPA_0: trough concentration of mycophenolic acid.

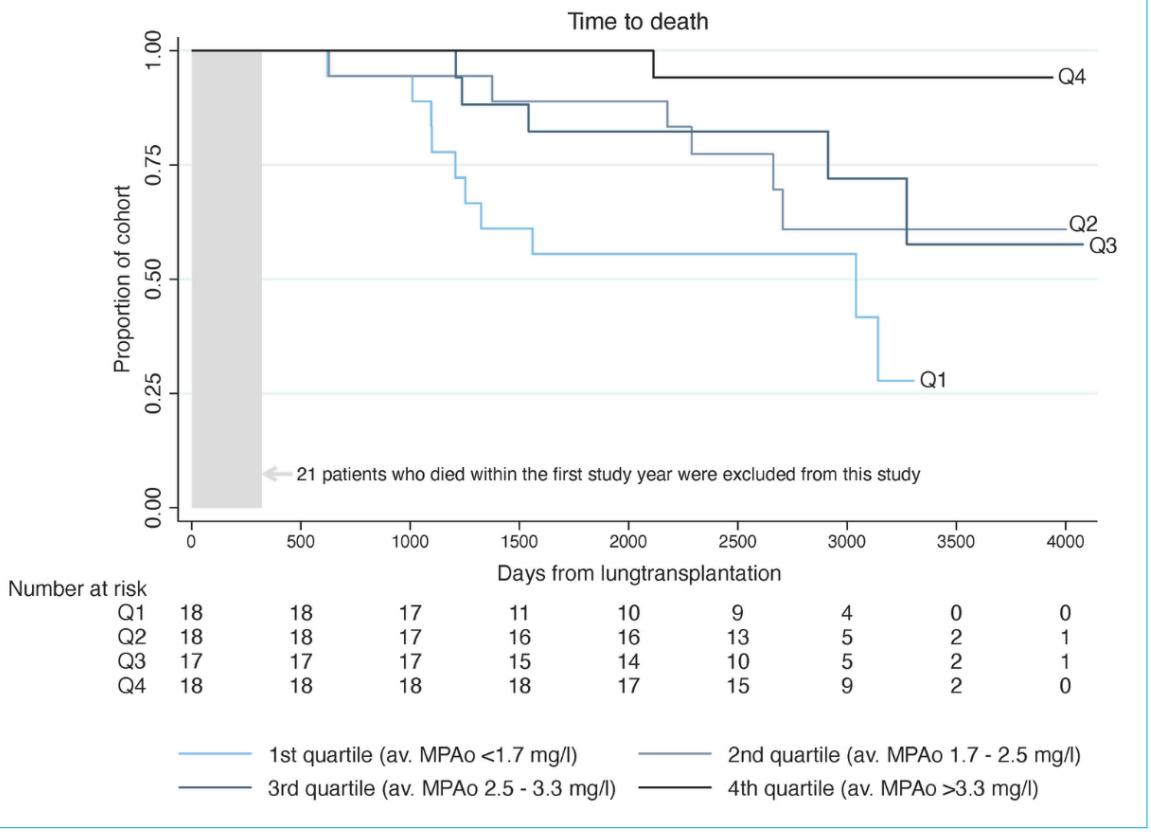


Table 2:
Univariable and multivariable event-time-analytical Cox proportional-hazards regression model in 71 patients with chronic lung allograft dysfunction (34 events) as the outcome.

		Time varying covariate, number of measurements*	Hazard ratio	95% confidence interval	p-value
Univariable analysis	MPA_0, mg/l	No	1.053	0.851–1.303	0.633
	MPA_0, mg/l*year	Yes, median of 9 (IQR 5–15) per patient	0.979	0.906–1.058	0.597
Univariable analysis	MMF dose, g	No	1.754	0.707–4.353	0.226
	MMF dose, g*year	Yes, median 45 (IQR 21–79) per patient	0.937	0.694–1.265	0.672
Multivariable analysis**	MPA_0, mg/l	No	1.062	0.773–1.418	0.756
	MMF dose, g	No	1.892	0.567–6.317	0.300
	Lymphocytes, G/l	No	0.590	0.179–1.941	0.385
	MPA_0, mg/l*year	Yes, median of 9 (IQR 5–15) per patient	0.986	0.898–1.082	0.764
	MMF dose, g*year	Yes, median 45 (IQR 21–79) per patient	0.877	0.602–1.277	0.495
	Lymphocytes, G/l*year	Yes, median 112 (IQR 56–181) per patient	1.074	0.809–1.425	0.621

CLAD:, chronic lung allograft dysfunction; IQR: interquartile range; MMF: mycophenolate mofetil; MPA_0: trough concentration of mycophenolic acid.

The global Schoenfeld residual test (p = 0.7501) suggested that the proportional-hazards assumption was not violated.

* For the time varying covariate, the interaction between time and the variable was modelled based on 18,431 different time intervals (each interval with a median time of 10 (IQR 2–24) days) clustered in 71 individuals over 382.97 patient-years.

** For the multivariable analysis there was no significant collinearity between MPA_0, MMF dose and lymphocytes (uncentred variance inflation factors between 2–5).

Table 3:
Chest computed tomography and transbronchial biopsy (with chronic lung allograft dysfunction group). Values are displayed as n (%).

CLAD stage	Patients with CLAD (n = 34)	Chest CT alterations (n = 29)	TBB (n = 15)	TBB alterations (n = 2)
1	11	10 (90.9%)	5	1 (30%)
2	12	9 (75%)	3	1 (20%)
3	7	6 (85.7%)	5	0 (0%)
4	4	4 (100%)	2	0 (0%)

CLAD: chronic lung allograft dysfunction; TBB: transbronchial biopsy; CT: chest computed tomography

Discussion

Early diagnosis and treatment of chronic lung allograft dysfunction are important prognostic factors for survival [2, 6]. In this study, no association could be established between MPA_0 or mycophenolate mofetil dosage and the occurrence of chronic lung allograft dysfunction or death. In addition, although patient age was classified as a risk factor for chronic lung allograft dysfunction, there was no association between age and MPA_0 or mycophenolate mofetil dosage.

According to the ISHLT's annual report, survival after lung transplantation is the shortest in patients with interstitial lung disease and COPD as underlying diseases and the longest in those with underlying cystic fibrosis [5, 7, 23]. Consistent with this finding, our study showed that chronic lung allograft dysfunction developed in only 15.4% of patients with cystic fibrosis as an underlying disease and in 69.6% of those with COPD and 50% of those with interstitial lung disease. We postulate that patients with COPD have an increased risk of developing chronic lung allograft dysfunction, whereas the risk is lower in patients with cystic fibrosis. This is influenced by the significantly higher age in the chronic lung allograft dysfunction group than in the cohort without chronic lung allograft dysfunction. In addition, increasing age, CT chest changes compatible with chronic lung allograft dysfunction, and chronic lung allograft dysfunction stages demonstrated a significant correlation.

For patients whose mycophenolate mofetil dose was individually adjusted depending on the mycophenolic acid AUC, Le Meur et al. showed fewer rejections in patients in the early stages after kidney transplant compared with a group of patients who received a fixed dose of mycophenolate mofetil [24]. In our lung transplant centre, MPA_0 and the number of lymphocytes were mainly used to optimise the mycophenolate mofetil dosage. AUC analysis is not performed for mycophenolic acid, for which reason we cannot relate our results to the mycophenolic acid AUC. Yabuki et al. found large variations in patients after solid organ transplantation [25]. In addition, they reported that the mycophenolic acid AUC showed only a weak correlation with MPA_0 or mycophenolate mofetil dosage [25, 26]. Considering the known wide variations in MPA_0, we measured lower lymphocyte counts with increasing MPA_0 and mycophenolate mofetil doses in our cohort, which indicated a dose-effect relationship. For this reason, we consider MPA_0 and the mycophenolate mofetil dosage to be suitable alternatives to the AUC for monitoring the mycophenolate mofetil effect.

The survival and frequency of patients with chronic lung allograft dysfunction in our cohort were comparable to those reported in other studies. Gallagher et al. analysed whether tacrolimus influences the development of chronic lung allograft dysfunction and reported chronic lung allograft dysfunction development and death rates of 44% and 34%, respectively [27]. In our cohort, 48% had chronic lung allograft dysfunction, and 32% of the patients died.

One of the limitations of the study is that in about 80% of the cases, no MPA_0 measurements were performed on the days on which an event (death, chronic lung allograft dysfunction) took place. In these cases, we considered the last MPA_0 measurement before the event in the model, as all patients have regular check-ups (a sensitivity analysis with the next measurement did not yield any different results). Furthermore, the study did not analyse any other immunosuppressant for the development of chronic lung allograft dysfunction, and only focused on the influence of mycophenolate mofetil. Since mycophenolate mofetil is only one component of the triple immunosuppressive therapy, our findings indicate that the net effect of mycophenolate mofetil alone is not of any significance for the onset of chronic lung allograft dysfunction. We suspect that chronic lung allograft dysfunction development is rather influenced by one of the other two components of the immunosuppressive therapy or by the overall effect of the triple immunosuppression. Gallagher et al. found that tacrolimus levels between 6 and 12 months after a lung transplant affected the development of chronic lung allograft dysfunction [1, 27]. Moreover, only cases of bilateral lung transplantation were included in the analysis. Our cohort also included re-transplantation (n = 3), in which the initial transplantation was performed before the start of observation in 2010. Another limitation is that antibody-mediated rejection and its relationship to mycophenolate mofetil dosage and MPA_0 were not investigated because they occur relatively rarely. Ultimately, the exclusion of 21 patients with a post-transplant survival of less than one year and of another 56 patients who did not provide general consent limited the study result and resulted in a smaller sample size, and due to the nature of our single-centre study we did not perform a sample size calculation.

We assumed that all patients had a constant risk of developing chronic lung allograft dysfunction over the entire observation period, regardless of the variables recorded. This “proportional hazards assumption” was checked using known risk factors for chronic lung allograft dysfunction and considered valid.

We are also aware that some of the patients had different histories and treatments before entering the study, which yielded an unequal patient population that could be only partially corrected using statistical methods. One of the strengths of this study is that the possible sources of error for the diagnosis of chronic lung allograft dysfunction were eliminated in accordance with the current ISHLT recommendations. Patients with other causes of a decrease in FEV1, such as lung infection, weight gain, or heart failure, were removed from the chronic lung allograft dysfunction group and assigned to the group without chronic lung allograft dysfunction [2]. Another strength is the long-standing, clearly defined guideline-based practice of immunosuppression therapy [20, 21].

The proven dose-effect relationship of MPA_0 and mycophenolate mofetil dose with the number of lymphocytes did not show any statistically significant association with chronic lung allograft dysfunction, and did not correlate with death. Thus, MPA_0 and mycophenolate mofetil dose are not primary factors related to rejection, but chronic lung allograft dysfunction may be influenced by other components of immunosuppression or other factors.

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Potential competing interests

All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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