TITLE PAGE

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- LT: Lung transplantation

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- ISHLT: International Society for Heart and Lung Transplantation
- BOS: Bronchiolitis obliterans syndrome
- RAS: Restrictive allograft syndrome
- FEV1: Forced expiratory volume in 1 second
- PGD: Primary graft dysfunction
- COPD: Chronic obstructive pulmonary disease
- ILD: Interstitial lung disease
- PAH: Pulmonary arterial hypertension

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Abstract page

Introduction: Azithromycin (AZI) may be an effective immune modulator in lung transplant (LT) recipients, and can decrease chronic lung allograft dysfunction (CLAD) rates, the leading cause of mortality after the first year post-LT. The aim of the study is to assess the effect of AZI initiation and its timing on the incidence and severity of CLAD in LT recipients.

Methods: Single-center retrospective study, including LT recipients from 01/01/2011 to 30/06/2020. Four groups were established: those who started AZI at the 3rd week post-LT (group A), those who received AZI later than the 3rd week post-LT and had preserved FEV1 (B), those who did not receive AZI (C) and those who started AZI due to a decline in FEV1 (D). The dosage of AZI prescribed was 250 mg three times per week. CLAD was defined and graduated according to the 2019 ISHLT criteria.

Results: We included 358 LT recipients: 139 (38.83%) were in group A, 94 (26.25%) in group B, 91 (25.42%) in group C, and 34 (9.50%) in group D. Group A experienced the lowest CLAD incidence and severity at 1 ($p = 0.01$), 3 ($p < 0.001$), and 5 years post-LT, followed by Group B. Groups C and D experienced a higher incidence and severity of CLAD ($p = 0.015$). Initiation of AZI prior to FEV1 decline (groups A and B) proved to be protective against CLAD after adjusting for differences between the treatment groups.

Conclusions: Early initiation of AZI in LT recipients could have a role in decreasing the incidence and severity of CLAD. In addition, as long as FEV1 is preserved, initiating AZI at any time could also be useful to prevent the incidence of CLAD and reduce its severity.

Main manuscript

Introduction

Lung transplantation (LT) is a well-established treatment option for patients with advanced respiratory diseases in whom all available treatment options have been optimized.[1] Despite advances made in different aspects of post-LT treatment, chronic lung allograft dysfunction (CLAD) continues to be one of the main factors limiting patient survival after the first year post-transplant.[2] The CLAD concept encompasses different clinical entities whose final outcome is a progressive loss of pulmonary function that leads to a reduced quality of life and results in limited survival. In recent years, different CLAD phenotypes have been defined according to their radiological and functional characteristics, with four groups being considered according to the consensus definition by the International Society for Heart and Lung Transplantation (ISHLT): bronchiolitis obliterans syndrome (BOS), restrictive allograft syndrome (RAS), mixed phenotype, and undefined phenotype.[3] This

classification has significant prognostic implications, as BOS, which is the most frequent phenotype, has a better prognosis than RAS.[4]

There are multiple known risk factors for the onset of CLAD, both immune-mediated and non-immune-mediated. Although some of the common pathophysiological pathways that lead to the changes in pulmonary architecture are known, the mechanisms explaining why BOS predominates in some patients and RAS in others remain undetermined.[5]

To date, no fully effective treatment for CLAD is known. However, several therapies have shown promise to slow the loss of lung function, including methotrexate, total lymphoid irradiation, extracorporeal photopheresis, thymoglobulin, montelukast, and, more recently, antifibrotics, such as nintedanib or pirfenidone.[6–15]

Azithromycin, a macrolide antibiotic that inhibits bacterial protein synthesis and reduces biofilm formation, is the most widely used and studied treatment for CLAD. In addition to its antibiotic properties in different infections, it has proven to be helpful as an anti-inflammatory and immunomodulator in different chronic inflammatory respiratory, dermatological, and genitourinary disorders with a predominantly neutrophilic inflammatory response.[16,17] Because neutrophilic airway inflammation is an essential non-alloimmune mediator in the onset of CLAD, azithromycin was introduced as a potential treatment of this condition in 2003.[18] In the following years, different studies analyzed the usefulness of this drug in patients with BOS, demonstrating a stabilization or improvement in pulmonary function.[19] Since then, different studies have demonstrated the usefulness of azithromycin in preventing CLAD and even improving post-LT survival.[20–22] Despite the above, azithromycin is not used as a standard treatment in all lung transplant units, and different centers start the treatment with this drug at different post-transplant times and at varying doses.

The aim of this study is to evaluate whether the timing of azithromycin initiation has an impact on the prevention of CLAD.

Material and Methods

It was a retrospective, single-center study including LT patients treated in Marqués de Valdecilla University Hospital of Santander (Spain) between 1 January 2011 and 30 June 2020.

Pre-transplant demographic characteristics (sex, age, and respiratory disease responsible for the transplant) were recorded for each patient. Variables related to the LT included the type of transplant (single or double), use of induction therapy, immunosuppressive maintenance treatment, and the presence of primary graft dysfunction according to the ISHLT classification 0, 24, 48, and 72 hours after the transplantation.[23] In addition, all episodes of acute cellular rejection, defined and graded according to the ISHLT Working Formulation, were recorded for each patient.[24]

The standard immunosuppression protocol applied in our center consists of an anti-calcineurinic (preferably tacrolimus), mycophenolate mofetil, and corticosteroids. Before 1 April 2016, induction with basiliximab (an interleukin-2 receptor antagonist) was exclusively used in selected cases, such as in patients with renal failure, recipients over 65 years of age, or in those in whom hemodynamic instability was expected during the immediate postoperative period (such as patients with pulmonary hypertension). Ever since, induction with basiliximab has been administered to all patients as per protocol.

As of January 1, 2017, per protocol, all transplant patients at our center received azithromycin 250 mg three times a week, starting in the 3rd week after transplant. From that same date, the patients transplanted before 01/01/2017 were started on azithromycin 250 mg three times a week in the successive check-ups, regardless of the time that had elapsed since the transplant or the clinical changes. Our study population was classified into four treatment groups:

- Group A: patients who started azithromycin at third week post-LT.

- Group B: patients who received azithromycin later than the third week post-LT and had preserved baseline forced expiratory volume in 1 second (FEV1).
- Group C: patients who did not receive azithromycin.
- Group D: patients who started treatment with azithromycin due to a loss of pulmonary function.

In order to establish the diagnosis of CLAD, only patients with at least 6 months of follow-up were included. The definition of chronic graft dysfunction was based on functional criteria, whereas the BOS, RAS, and mixed phenotypes were established according to functional and radiological criteria in accordance with the ISHLT consensus.[3] There were two independent evaluators to determine the diagnosis of CLAD, and these evaluators were blinded of each patient's azithromycin group. Disagreements in CLAD onset were resolved by consensus between the two investigators who made the CLAD diagnoses with a third investigator keeping the blind.

According to our center's protocol, a bronchoscopy with transbronchial biopsy is performed in all patients approximately 2–3 weeks after LT. As per protocol, a transbronchial biopsy is not performed during the subsequent follow-up, and the conduct of this procedure is limited to those cases in which there is a clinical, radiological, or functional suspicion of acute rejection.

In case of an acute cellular rejection ≥A2 according to ISHLT Working Formulation, high-dose methylprednisolone treatment is administered at a dosage of 10–15 mg/kg/day for three days, followed by a subsequent tapered corticosteroid regimen, and a new follow-up transbronchial biopsy is repeated around 3–6 weeks later.

The study was approved by the Drug Research Ethics Committee of Cantabria (Spain) and coordinated by the Valdecilla Research Institute (IDIVAL, *Instituto de Investigación Valdecilla*), with protocol code 2021.341.

Statistical Analysis

Software IBM SPSS Statistics 20 was used to perform the statistical analyses. Continuous quantitative variables were expressed as a mean ± standard deviation in the case of those following a normal

distribution, and as a median and interquartile range in the case of those not following this type of distribution. On the other hand, categorical variables were expressed as frequencies and percentages.

The Smirnov-Kolmogrov test was used to determine whether or not the continuous quantitative variables followed a normal distribution. Student's t-test for independent samples was used to analyze differences between a quantitative variable following a normal distribution and a qualitative one. In contrast, Mann-Whitney U test was used to compare quantitative variables not following a normal distribution with qualitative variables. The chi-squared test was used to explore the association between two qualitative variables. A log-rank test was carried out to study the event-free period (onset of CLAD), and the cause-specific Cox proportional hazards model with graft loss/death events right censored was used for both univariable and multivariable analysis by using forward stepwise regression with a threshold of p <0.20. In all cases, a p-value ≤0.05 was considered to be statistically significant.

Results

From an initial cohort of 393 patients, 358 patients who had been followed for at least six months to establish the diagnosis of CLAD were included in the study. Of these, 64.2% were men and most of them (68.7%) had undergone double lung transplantation. A summary of the population characteristics is included in Table 1.

A total of 139 (38.8%) of these patients had received azithromycin from the third week following the LT (group A), 94 (26.2%) received azithromycin later than the third week post-LT without having a decline in their FEV1 (group B), 91 (25.5%) did not receive azithromycin (group C), and 34 (9.5%) received the drug due to a loss of pulmonary function (group D).

The characteristics of the patients included in the different azithromycin treatment groups were similar in terms of their age, sex distribution, type of anti-calcineurinic agent used, and respiratory disorder responsible for the transplantation. However, the incidence of single lung transplantations was greater among those patients who did not receive azithromycin (group C) compared with the rest of them (C = 45.1% vs. A = 25.2%, B = 28.7%, and D = 26.5%; p = 0.011). The incidence of induction therapy was greater in the group of patients who received azithromycin at the 3rd week

post-transplant (group A) (A = 100% vs. B = 19.1%, C = 18.7%, D = 20.6%; p <0.001), as was that of primary graft dysfunction (A = 30.2% vs. B = 12.8%, C = 16.5%, D = 11.8%; p = 0.003).

A total of 201 (56.1%) patients developed acute cellular rejection during the follow-up period. Among these, 153 (76.1%) cases developed rejection prior to being discharged following the transplantation, and 48 (23.9%) experienced rejection during the follow-up period. The median number of episodes of acute cellular rejection per patient was 1 (0–2). Patients included in treatment group A experienced the least number of acute rejections during the follow-up period compared with the other groups (0 [0–1] in group A vs. 1 [0–2] in groups B and C vs. 0.75 [1–2] in group D; p <0.001).

Over a mean follow-up period of 4.16 (2.35–6.50) years, a total of 108 patients (30.2%) of the case series developed CLAD, 91 (84.3%) of whom had the BOS phenotype, 14 (13.0%) had the RAS phenotype, and 3 (0.8%) had a mixed phenotype.

The incidence of CLAD among the patients who started treatment with azithromycin without having a decline in their FEV1 (groups A and B) was significantly lower than among those who did not take azithromycin (group C) (21.5% vs. 33.0%; p = 0.023).

Patients in group A experienced the fewest episodes of CLAD at the first, third, and fifth-year post-transplant compared with the other groups, followed by groups B, C, and D, respectively (Table 2).

In addition to having a lower incidence of CLAD, patients in group A experienced lower severity of CLAD (only CLAD stages 1 and 2), while those who did not receive azithromycin or those who received it due to a decline in their FEV1 (groups C and D) experienced higher severity of CLAD (stages 3 and 4) (Table 3; Figure 1).

Because patients in group A had a significantly shorter follow-up time with a median of 3.39 years, and this could generate a bias, a Cox regression was performed to assess which variables influenced the development of CLAD, truncating the follow-up to 3 years. In the univariate analysis, the time of the transplant, induction with basiliximab, the different groups of azithromycin and the number of

cellular rejections during follow-up had statistical significance. However, in the multivariate analysis, only the number of acute cellular rejections was an independent risk factor for the development of CLAD (hazard ratio [HR] = 1.393; 95% confidence interval [CI] 1.154–1.683; *p* = 0.001), and receiving azithromycin from the 3rd week (group A) (HR = 0.243; 95% CI 0.108 - 0.546; p = 0.001) and the use of azithromycin beyond the 3rd week without a fall in FEV_1 (group B) (HR = 0.345; CI 95% 0.173 - 0.685, *p* = 0.002) were independent protective factors for CLAD development. (Table 4, figure 2).

Discussion

The findings of this study show how the time of initiation of azithromycin prophylaxis is relevant in preventing the onset of CLAD in a cohort of LT patients. Those starting treatment at the third week post-transplant had a significantly lower incidence of CLAD at 1-, 3-, and 5-years post-transplant compared to the rest, in addition to lower severity of CLAD and fewer episodes of acute cellular rejection. As long as FEV1 is preserved, we found that it is never too late to start azithromycin prophylaxis for CLAD prevention. Group B patients, who had a preserved FEV1 but started later than the third week post-LT, experienced the second highest rates of CLAD prevention and lower severity during follow-up. In addition, the start of azithromycin without a fall in FEV1, either in the 3rd week or at follow-up (groups A and B), was the only protective factor for the development of CLAD in the first 3 years in the multivariate analysis.

Prevention of CLAD is one of the main objectives in the follow-up of LT patients, as it is the leading cause of death after the first year. Despite knowing its risk factors, CLAD is difficult to predict and treat. Although different pathways have been explored, to date there is no sufficiently validated tool or biomarker available to predict it. Fortunately, different therapies tested in small case series have been shown to reduce the rate of decline in pulmonary function. In addition to conventional immunosuppression regimes, one of the most widely studied drugs that has proven to have beneficial effects on CLAD in LT patients is azithromycin.

However, there is no consensus on when this treatment should be started, at what dose, or for which indications. Multiple indications for initiating azithromycin post-LT have been studied, including primary prophylaxis, or after pulmonary function begins to decline, or after an adverse

event that increases the risk of developing CLAD (such as lymphocytic bronchiolitis or neutrophilia in bronchoalveolar lavage [BAL] sample).[25]

The findings of different studies demonstrate how treatment with azithromycin can slow the loss of pulmonary function in LT patients who have already developed CLAD and that early initiation of this drug contributes to preserving pulmonary function compared to those who start the treatment at a later date or who take a placebo.[26–28]

The use of azithromycin as prophylaxis for CLAD was studied by Vos *et al*. in a clinical trial whose findings demonstrated a beneficial effect in preventing the onset of CLAD in LT patients. However, no effect on overall survival was found, probably due to the short follow-up period and small sample size.[20] A follow-up of this study was published by Ruttens *et al*., who demonstrated that the prophylactic use of azithromycin was beneficial in preventing the onset of CLAD without significant associated adverse effects.[21] The use of azithromycin could also be beneficial in preventing the onset of CLAD associated with environmental pollution, as demonstrated in a European multicenter study.[29] In contrast, the findings of a study carried out by Van Herck *et al*. failed to demonstrate the benefit of azithromycin on early lung function following a transplantation.[30]

A recent experience reported by Li *et al*. revealed that the prophylactic use of azithromycin benefited overall survival after LT, even though it was not found to be useful in preventing CLAD in the adjusted analysis.[22]

Regarding the treatment's safety, one of the main concerns are the adverse effects associated with the long-term use of azithromycin, as it has been linked to hearing loss, bacterial resistance, arrhythmias, and even deaths of cardiovascular cause.[31,32] However, numerous studies that have already been cited in this paper, in which azithromycin was used in LT patients, did not report significant cardiovascular adverse effects.

Group B patients, who had a preserved FEV1 but started later than the third week post-LT, experienced the second lowest rates of CLAD and lower severity during follow-up.

Regarding acute cellular rejection, acute cellular rejection was the only independent risk factor for CLAD development in multivariate analysis. Patients on azithromycin experienced significantly fewer

acute rejections than those who did not receive azithromycin, although this is not a currently known beneficial effect of prophylaxis with this drug. One potential explanation for this effect are the known benefits of azithromycin as an anti-inflammatory and immunomodulator in the airways.[16,33] A recent experience showed that microbiome dysbiosis in LT patients increased the risk of acute cellular rejection episodes, although chronic treatment with azithromycin was not responsible for changes in the composition of this microbiome.[34]

Finally, it must be considered that patients in group A belong to a more recent transplant era and have less follow-up time. A potential "era" effect of the transplant may influence the results. However, the only change made in the immunosuppression and prophylaxis protocol in the study period was the introduction of azithromycin and the induction of basiliximab. Although in the univariate analysis both induction and the era of transplant were factors related to the development of CLAD at 3 years, neither and only the azithromycin groups had statistical significance in the multivariate analysis.

The findings of our study should be viewed with caution, as this is a retrospective single-center, creating a classification bias. The patients treated with azithromycin due to experiencing a decline in their FEV1 had a higher incidence of CLAD, since treatment of worsening lung function was the indication for azithromycin and not prophylaxis. In addition, the effect of azithromycin in preventing different CLAD phenotypes was not examined due to the small number of RAS-type and mixed phenotype CLAD events. Although known risk factors for CLAD, such as primary graft dysfunction, type of anti-calcineurinic agent used, or acute cellular rejections were included in our analyses, others such as episodes of viral or bacterial infections, gastroesophageal reflux, alloimmunity, or cytological analyses of bronchoalveolar lavage samples were not included. Furthermore, we failed to explore the potential adverse effects associated with the use of this drug, patients who discontinued azithromycin and their reasons, and the time from transplantation to the start of azithromycin in group B and the total time of azithromycin treatment for each patient were not collected.

All in all, the results obtained from this retrospective cohort of LT patients indicate that the early use of azithromycin can be beneficial in preventing the onset of CLAD. But it is never too late to initiate this treatment in patients with a preserved pulmonary function, in whom it has also been shown to be linked to a longer CLAD-free time compared with patients who have not received it.

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Table 1. Baseline population characteristics

		At the 3rd week (GROUP A)	After the 3rd week and had preserved FEV1 (GROUP B)	Not treated with azithromycin (GROUP C)	Treated due to a decline in FEV1 (GROUP D)	*p*
n	358	139 (38.83%)	94 (26.25%)	91 (25.42%)	34 (9.50%)	-
Age at transplantation (years)	58.26 (51.81–62.28)	59.01 (50.49–62.25)	57.20 (51.47–61.67)	59.52 (53.09–63.61)	57.02 (52.98–60.53)	0.293
Period of transplant						
- 01/01/2011 – 31/12/2015	180 (50.3%)	0 (0%)	76 (80.9%)	78 (85.7%)	26 (76.5%)	< 0.001
- 01/01/2016 – 30/06/2020	178 (49.7%)	139 (100%)	18 (19.1%)	13 (14.3%)	8 (23.5%)	
Sex						
- Male	230 (64.2%)	89 (64%)	61 (64.9%)	58 (63.7%)	22 (64.7%)	0.998

- Female	128 (35.8%)	50 (36%)	33 (35.1%)	33 (36.3%)	12 (35.3%)	
Type of transplant						
- Single lung	112 (31.3%)	35 (25.2%)	27 (28.7%)	41 (45.1%)	9 (26.5%)	0.011
- Double lung	246 (68.7%)	104 (74.8%)	67 (71.3%)	50 (54.9%)	25 (73.5%)	
Lung diseases						
- COPD	123 (34.1%)	40 (20.8%)	39 (41.5%)	28 (30.8%)	15 (44.1%)	
- ILD	165 (46.1%)	64 (46%)	41 (43.6%)	46 (50.5%)	14 (41.2%)	
- Bronchiectasis	13 (9.2%)	19 (13.7%)	7 (7.4%)	5 (5.5%)	2 (5.9%)	0.582
- PAH	12 (3.4%)	5 (3.6%)	3 (3.2%)	3 (3.3%)	1 (2.9%)	
- Other	22 (6.1%)	10 (7.2%)	3 (3.2%)	7 (7.7%)	2 (5.9%)	
- Re-transplantation	4 (1.1%)	1 (0.7%)	1 (1.1%)	2 (2.2%)	0 (0%)	
Anti-calcineurin agent						
- Tacrolimus	315 (89%)	129 (92.8%)	83 (89.2%)	73 (83.0%)	30 (88.2%)	0.147
- Cyclosporine	39 (11%)	10 (7.2%)	10 (10.8%)	16 (16.5%)	4 (11.8%)	
Induction (basiliximab)	181 (50.6%)	139 (100%)	18 (19.1%)	17 (18.7%)	7 (20.6%)	<0.001
PGD	73 (20.4%)	42 (30.2%)	12 (12.8%)	16 (16.5%)	4 (11.8%)	0.003
Acute cellular rejection per patient	1 (0–2)	0 (0–1)	1 (0–2)	1 (0–2)	1 (0.75–2)	<0.001
1-year survival	92.2%	96.4%	100%	74.7%	0%	<0.001
5-year survival	65.2%	74.5%	87.2%	30.8%	73.5%	<0.001
Follow-up time (years)	6.01 (3.82 – 8.52)	3.39 (2.54 – 4.09)	7.21 (6.10 – 8.82)	8.47 (6.63 – 9.69)	8.44 (6.48 – 10.30)	<0.001
CLAD at time of analysis	108 (30.2%)	12 (8.63%)	38 (40.42%)	30 (32.96%)	28 (82.35%)	<0.001

CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; PGD, primary graft dysfunction.

Table 2. prevalence of CLAD at 1, 3 and 5 years after lung transplantation

	At the 3rd week (GROUP A)	**After the 3rd week and had preserved FEV1**	**Not treated with azithromycin**	**Treated due to a decline in FEV1**	**Log-rank**

		(GROUP B)	(GROUP C)	(GROUP D)	
n	139 (38.83%)	94 (26.25%)	91 (25.42%)	34 (9.50%)	-
1 year	1.5%	2.1%	7.8%	11.8%	0.010
3 years	8.5%	15%	31.5%	52.9%	<0.001
5 years	17.4%	28.1%	45.9%	76.5%	<0.001

Table 3. CLAD degrees in the different azithromycin treatment groups

	CLAD	**CLAD 1**	**CLAD 2**	**CLAD 3**	**CLAD 4**	***p***
Group A	12/139	8 (66.7%)	4 (33.3%)	0 (0%)	0 (0%)	0.015
Group B	38/94	14 (36.8%)	12 (31.6%)	7 (18.4%)	5 (13.2%)	
Group C	30/91	16 (53.3%)	5 (16.7%)	4 (13.3%)	5 (16.7%)	
Group D	28/34	6 (21.4%)	4 (14.3%)	9 (32.1%)	9 (32.1%)	

Table 4: Univariate and multivariate analysis for the development of CLAD at 3 years post-transplantation

	Univariate			Multivariate		
	HR	**95% CI**	***p*-value**	**HR**	**95% CI**	***p*-value**
Age at transplantation	1.002	0.978 – 1.027	0.871			
Gender						
- Male	Ref.	-	-			
- Female	0.771	0.448 – 1.326	0.347			
Type of transplant						
- Single lung	Ref.	-	-	Ref.	-	-
- Double lung	0.614	0.367 – 1.027	0.063	-	-	-
Period of transplantation						
- 01/01/2011 – 31/12/2015	Ref.	-	-	Ref.	-	-
- 01/01/2016 – 30/06/2020	0.380	0.217 – 0.665	0.001	-	-	-
Underlaying disease						
- COPD	Ref.	-	-			
- ILD	1.055	0.601 – 1.849	0.853			
- Bronchiectasis	0.491	0.146 – 1.646	0.249			
- PAH	1.664	0.496 – 5.579	0.410			
- Retransplantation	1.024	.259 – 14.306	0.523			
- Others	1.047	0.359 – 3.049	0.933			
Basiliximab induction	0.406	0.234 – 0.705	0.001	-	-	-
Anticalcineurinic						
- Cyclosporine	Ref.	-	-			
- Tacrolimus	0.920	0.396 – 2.138	0.847			
PGD	0.786	0.399 – 1.547	0.786			
Azithromycin groups						

- No azithromycin	Ref.	-	-	Ref.	-	-
- At 3rd week (A)	0.197	0.090 – 0.434	< 0.001	0.243	0.108 – 0.546	0.001
- After the 3rd week and preserved FEV_1 (B)	0.371	0.187 – 0.737	0.005	0.345	0.173 – 0.685	0.002
- Due to FEV_1 decline (D)	1.666	0.881 – 3.152	0.117	1.553	0.821 – 2.939	0.176
Acute cellular rejection before discharge at transplantation	1.384	0.830 – 2.306	0.213			
Nº of acute cellular rejections	1.541	1.283 – 1.852	< 0.001	1.393	1.154 – 1.683	0.001

COPD = Chronic Obstructive Pulmonary Disease; ILD = Interstitial Lung Disease; PAH = Pulmonary Arterial Hypertension; FEV1 = Forced Expiratory Volume in the first second; PGD = Primary Graft Dysfunction; CI = confidence interval; HR = hazard ratio.

Figure 1. Distribution of CLAD stages in the different treatment groups. Group A patients are those with milder CLAD stages, while group D patients are those with more severe CLAD stages (p = 0.015).

Figure 2. Cumulative Cause-Specific Hazard for CLAD at 3-year post-transplant follow-up