Evaluating Novel Prognostic Markers and Preventative Therapies for Chronic Lung Allograft Dysfunction after Lung Transplantation

by

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ABSTRACT

Introduction: Survival following lung transplantation remains limited by chronic lung allograft dysfunction (CLAD). Few therapeutic options have been shown to be effective in established CLAD thus early detection and prevention are key.

Objective: We aimed to identify potential early prognostic radiographic markers on lung ventilation-perfusion (VQ) scans and evaluate our center's strategy of using azithromycin prophylactically to prevent CLAD.

Methods: Retrospective cohort studies were conducted using prospectively collected data from the University of Alberta lung transplant program databases on patients transplanted between January 1, 2004, and December 31, 2016, in conjunction with supporting data from clinical databases to address the questions of interest. Inclusion was boundaried at 2016 to allow a minimum of 5 years of follow-up to facilitate risk of primary outcome of interest, CLAD. Inclusion criteria were all adult double lung transplants with sufficient data for each study; single lung, heart-lung, and living lobar lung transplants were excluded given some outcomes of interest are not defined in these population. PGD and CLAD grades were defined per consensus guideline definitions and an additional syndrome of baseline lung allograft dysfunction (BLAD) was defined as per our published definition of failure to achieve spirometry measures of at least 80% predicted on 2 consecutive tests at least 3 weeks apart. In part i) we examined how mismatched perfusion defects detected on VQ scans affected survival and in part ii) we assessed the prognostic implications of abnormal left-right lung perfusion differential on VQ scans. In part iii), we examined the association between use of azithromycin prophylaxis and survival and lung function.

Results: i) 169/340 patients (49%) had a relative perfusion differential > 10% on 3-months VQ scan. Patients with increased perfusion differential had increased risk of death or retransplantation (p=0.011) and of CLAD onset (p=0.012) after adjustment for other radiographic/endoscopic abnormalities; ii) 35/373 patients (9%) had VQ scans with perfusion defects. Patients with PD had similar 1-year survival (100% vs. 98%, p=1.00), overall survival (log rank p=0.90) and peak FEV1% predicted (94% [SD 20%] vs. 92% [SD 21%]; p=0.58). Anticoagulation did not affect these relationships; iii) 344/445 patients (77%) received azithromycin prophylaxis [median time from transplant 51 days]. Azithromycin prophylaxis was associated with improved survival (hazard ratio [HR] 0.60 [95% confidence interval [CI] 0.44-0.84]; p=0.002) in our adjusted model and with reduced unadjusted risks of CLAD onset (HR 0.64 [95% CI 0.44-0.94]; p=0.025) and BLAD (odds ratio 0.55 [95% CI 0.35-0.86]; p=0.009).

Conclusion: i) Wide left-right lung perfusion differential was associated with increased adjusted risk of death and CLAD onset; ii) mismatched perfusion defects on lung VQ scans were not associated with survival; iii) azithromycin prophylaxis was associated with improved survival after lung transplant, potentially through reducing BLAD risk.

PREFACE

This thesis represents original work by David Li. Many collaborators have contributed to each chapter as detailed below. Chapters 3 and 4 have been published in peer reviewed journals. Chapter 2 is currently accepted to *Transplantation* journal in manuscript form.

The research projects that this thesis is comprised of received research ethics approval from the University of Alberta Institutional Review Board (Pro00095903).

The following are the publications and their associated chapters:

Chapter 2:

Li D, Abele J, Sunner P, Kapasi A, Hirji A, Weinkauf J, Lien D, Varughese R, Nagendran J, Halloran K. (Accepted). Relative lung perfusion on ventilation-perfusion scans after double lung transplant. *Transplantation*.

Chapter 3:

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Chapter 4:

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associated with Improved Overall Survival. *The Journal of Heart and Lung Transplantation*. 10.1016/j.healun.2020.09.006.

For each of these three studies I had responsibilities of: conceptualization, data collection, participation in formal analysis, and writing of the primary manuscript draft.

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CHAPTER 1 – Introduction

Lung transplantation is the only definitive treatment for many end stage lung diseases. Despite continual advancements since the first successful case 40 years ago[1], outcomes following lung transplant remain the most challenging of all solid organ transplants with median survival after double lung transplant of approximately 8 years internationally.[2] A key reason for this is that the lungs are unique among transplantable solid organs as a highly immunogenic site that is directly exposed to the external environment.[3] This juxtaposition complicates an already fine balance between maintaining sufficient immunosuppression to avoid rejection and managing infections and other complications of immunosuppression in the post-transplant period.

This introductory chapter will briefly review the most important forms of allograft dysfunction following lung transplant – with particular focus on chronic lung allograft dysfunction. It will aim to highlight some of the existing gaps in knowledge to help contextualize the series of studies that this thesis is comprised of.

Chronic lung allograft dysfunction – common and deadly

At 5 years post-transplant, approximately half of all lung transplant recipients will develop chronic lung allograft dysfunction (CLAD), or progressive loss of lung allograft function[4]. It is defined clinically as a persistent decline (>3 months without recovery) in the key lung function metric – forced expiratory volume in one second [FEV1] to less than 80% of a patient's personal baseline, which is itself defined as the average of the two best FEV1 values they achieved post-transplant that are at least 3 weeks apart, after ruling out

confounders for the lung function decline.[4] Despite many advances in lung transplantation, CLAD remains the most important limiter of long-term survival following lung transplant after the first post-transplant year.[4,5]

CLAD is an inclusive term encompassing previously identified chronic dysfunction phenotypes as well as those more recently described. The first described CLAD phenotype was bronchiolitis obliterans syndrome (BOS), a physiologically observed surrogate intended to diagnose inflammatory and fibrotic remodeling processes in the small airway, obliterative bronchiolitis (OB).[6,7] Later investigations revealed a distinct CLAD phenotype designated restrictive allograft syndrome (RAS) characterized by a restrictive pattern on spirometry and lung volume measurements with scarring changes on imaging and inflammation and fibrosis of the lung parenchyma on histology.[7,8] Further studies revealed mixed phenotypes of CLAD with evidence of lung parenchymal fibrosis but non-restrictive pattern on spirometry.[4,9] As it stands, CLAD appears to be a heterogeneous clinical entity which constitutes a “final common pathway”, with clear links to alloimmune/rejection processes including those cellular or antibody mediated, but also with demonstrated associations with non-alloimmune drivers such as repeat lung injury from chronic respiratory infections and gastroesophageal reflux micro-aspiration as well.[4]

The search for effective CLAD therapies

Despite its deadly implications, there remain limited therapeutic options for established CLAD. Several strategies have been tried targeting rejection, including changes in maintenance immunosuppression regimen, rescue cytolytic therapy with monoclonal

antibodies, extracorporeal photopheresis, and total lymphoid irradiation.[4,10] The results of these attempts have generally been disappointing with some stabilization of lung function shown with therapies such as azithromycin and montelukast but no reliable demonstrated survival benefit, and with the potential complications of treatment toxicity and infection.[4,11,12] Azithromycin, a macrolide antibiotic with known airway anti-inflammatory effects even at subtherapeutic antimicrobial dosing, has shown the most promise as a CLAD therapy.[13–16] A small, single-center, randomized trial showed that azithromycin treatment in CLAD patients improved FEV1 relative to placebo and metanalyses seem to support this finding.[15,16]

The critical role of early detection and prevention

As with many disease processes, earlier detection of CLAD may provide a window for prompt optimization of contributing factors or yield better outcomes from more timely initiation of treatment. This has remained challenging due to the heterogeneity of CLAD. Biomarkers from tissue biopsies, bronchoalveolar lavage sampling, and serum offer promise especially with advances in molecular methods of analysis, but none are currently established[17]. An early radiologic marker of CLAD would be useful as imaging is typically non-invasive and amenable to serial studies.[18,19] Air trapping on CT lung scans is known to be an imaging correlate of CLAD but the timing of this finding generally coincides with or follows CLAD onset.[20] The goal then is to identify radiologic changes that precede overt decline in lung function. While CLAD is thought primarily to be a disease of the airways, the pulmonary vasculature has received more attention with a recent study showing pulmonary vasculature volume from machine learning evaluation of

CT scans to be a strong prognostic marker of graft failure.[21,22] One readily accessible and relevant imaging study which does not currently see regular use in post-transplant evaluation is the lung ventilation-perfusion (VQ) scan.[23,24] Lung VQ scans are comprised of a pair of nuclear imaging tests in which radioisotopes are used to measure ventilation and blood flow in the lungs. These scans can plausibly offer both qualitative as well as quantitative information about airway and pulmonary vasculature function. Our program performs routine VQ scans at 3 months post-transplant to establish baseline function and sometimes note abnormalities, including mismatched perfusion defects and unbalanced right-to-left whole lung perfusion. This presents an opportunity to investigate the future implications of these changes.

In light of the limited options for treatment and early detection that are currently available, prevention becomes a priority. As noted, azithromycin has shown potential as a treatment for CLAD but a small, single-center randomized controlled trial showed that low dose azithromycin initiated shortly post-transplant improved CLAD-free survival as compared to placebo.[25] However, the use of a composite endpoint for sufficient power and the lack of a direct survival benefit limited the uptake of the strategy among transplant programs. Post hoc analysis of the original trial supported the initial findings but could not show a direct survival benefit either, though again constrained by sample size and follow-up duration.[26] Our program implemented the prophylactic azithromycin strategy shortly after the trial results were published in 2010 and as such have accumulated a large cohort of patients who have received the therapy along with a historical control group transplanted prior to 2010 who have not received it. We are thus well-positioned to comprehensively

evaluate the association between prophylactic azithromycin use and overall post-transplant survival as well as graft dysfunction.

Other important forms of lung allograft dysfunction

Though the primary focus of this thesis is on CLAD, there is a complex interplay between multiple forms of lung dysfunction after lung transplantation. These entities – primary graft dysfunction and baseline lung allograft dysfunction – can influence both survival and CLAD risk, and as such are important to study in parallel in lung transplant populations.

Primary graft dysfunction (PGD)

While chronic lung allograft dysfunction is the primary chronic form of lung dysfunction, PGD is the primary acute, immediate post-transplant form. PGD is a severe implantation response that occurs within 72 hours of transplantation, characterized by edema in the transplanted lungs with resultant hypoxemia. Clinically, the syndrome is defined by the presence of bilateral radiographic infiltrates in the allograft consistent with pulmonary edema and graded based on the ratio between the partial pressure of oxygen in arterial blood (PaO2) and the fraction of inhaled oxygen (FiO2) required on the ventilator (P/F ratio).[27] The designation is made after exclusion of confounders such as hyperacute rejection, cardiogenic edema, pneumonia, and vascular anastomotic obstruction. Grade 3 PGD (PGD3) is the most severe form of the syndrome and corresponds to a P/F ratio of less than 200 in the presence of pulmonary edema. PGD3 is strongly associated with mortality, especially if it is present in the 48-72 hours post-transplant period.[28,29]

Estimates of the incidence of PGD vary between centers but are typically cited as a 20-30% risk of PGD3 at any time within the first 72 hours.[28–30] The incidence of PGD3 in the 48–72 hour post-transplant period, where the implications are felt to be most severe and where much of the research efforts have focused, has been reported to be between 10-25%.[28–31]

The pathogenesis of PGD is complex but thought to relate to a cascade of changes that occur with ischemia then reperfusion of the transplanted lungs. The overall result is an inflammatory storm with disruption to the endothelium and epithelium of the allograft.[32] A number of donor and recipient risk factors have been associated with PGD risk. Established donor risk factors include older age and a history of cigarette smoking.[28,32] Procedural factors such as prolonged graft ischemic time and the use of cardiopulmonary bypass (CPB) intraoperatively also increase PGD risk.[33] Recipient risk factors include elevated BMI as well as preexisting left ventricular diastolic dysfunction and elevated mean pulmonary artery pressure.[31,34,35] The recipient's underlying lung disease also relates to PGD risk, with pulmonary arterial hypertension conveying heightened risk likely via elevated pulmonary artery pressures and traditional need for CPB perioperatively.[36] Interstitial lung diseases also appear to be associated with increased PGD risk, while COPD seems to be protective, which we have previously shown may relate to a size matching effect.[37,38]

PGD treatment remains largely supportive with use of modified lung protective ventilation leveraging positive end expiratory pressure, cautious diuresis, and use of inhaled

pulmonary vasodilators such as nitric oxide. Extracorporeal membrane oxygenation (ECMO) is used as a last resort to support patients with severe PGD.[30,32] Although PGD has been shown to involve acquired surfactant deficiency, exogenous surfactant administration remains experimental.[39]

Finally, PGD has long term consequences. A number of studies have shown associations between PGD and the risk of future development of CLAD[40–43], though our own analysis of this did not bear this association.[44] Our own analysis showed that PGD increases the risk of baseline lung allograft dysfunction (BLAD), where lung function fails to reach normal thresholds (see below). Other studies have showed that PGD continues to be associated with worse survival even beyond the first post-transplant year.[45]

Baseline lung allograft dysfunction (BLAD)

While CLAD describes a condition where lung function irreversibly declines from a recipient's personal post-transplant baseline, there is another group of patients do not attain a normal level of baseline lung function to begin with, irrespective of any decline. Our group originally described this form of lung dysfunction, which we termed baseline lung allograft dysfunction (BLAD) and showed that this was associated with an increased risk of death in a dose-dependent fashion with risk rising inversely related to the level of lung function achieved.[4,44,46]

We defined BLAD as failure to achieve a forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) on spirometry testing of at least 80% of the standardized,

population-derived reference value.[46] Notably, a wide range of factors may lead to poor baseline function, including causes both intrinsic and extrinsic to the lungs, such as diaphragmatic hemiparesis and large airway disease or dysfunction. Nevertheless, the separate designation of BLAD provides an important dimension to the evaluation of post-transplant graft function which helps complete the picture alongside CLAD.

Experimental logic and hypotheses

We have outlined that identifying early markers for allograft dysfunction and potential preventative therapies for CLAD are priorities in the current landscape of lung transplantation. As such, we sought to evaluate the following hypotheses, which will be presented in the three chapters that follow.

1. A novel imaging marker of unbalanced right-to-left whole lung perfusion detected on routine 3-months post-transplant VQ scans would be associated with increased risk of developing CLAD and future graft loss.
2. Mismatched perfusion defects, commonly associated with pulmonary emboli, seen on the same 3-months post-transplant VQ scans confer a benign prognosis in the context of routine post-transplant studies irrespective of therapeutic anticoagulation.
3. Chronic azithromycin therapy initiated prophylactically prior to CLAD onset would be associated with improved survival and reduced rates of CLAD and BLAD.

CHAPTER 2 – Relative lung perfusion on ventilation-perfusion scans after double lung transplant

INTRODUCTION

Imaging and functional assessments of the transplanted lung are important in the follow-up of patients after lung transplantation to establish baseline values and detect complications. High resolution computed tomography (CT) scans of the lungs and spirometry are mainstays of post-transplant evaluation to assess lung structure and physiology.[1] Ventilation-perfusion (VQ) scans are a promising complementary imaging modality that is relevant and accessible. While most often used to diagnose pulmonary thromboembolism, VQ scans have recognized value in the post-transplant evaluation of recipients, including detecting changes which may predate the decline in spirometry that is the hallmark of chronic lung allograft dysfunction (CLAD).[2–4]

Relative lung perfusion differential on VQ scans assesses the symmetry of pulmonary blood flow between the right and left lung, as well as indirectly assessing ventilation due to hypoxic pulmonary vasoconstriction response.[5] A right-to-left perfusion differential of less than 10% (55%-45%) is typically considered normal but the physiologic correlates and prognostic associations of perfusion differentials have not been studied.[6,7]

Our program performs routine VQ scans at 3-months post-transplant. Our objective was to characterize the population perfusion differential, the relationship to ventilation and lung function, and its utility as a marker of future risk of graft loss, chronic lung allograft

dysfunction (CLAD) onset, and baseline lung allograft dysfunction (BLAD).[8] We hypothesized that a wide perfusion differential would be common, associated with lower lung function at time of scan and associated with increased risk of future graft loss, CLAD and BLAD.

METHODS

Study Population

We conducted a retrospective cohort study of all adult patients who underwent double lung transplant in the University of Alberta lung transplant program between January 1, 2005, and December 31, 2016, with available 3-months post-transplant VQ scans. Inclusion was bounded at this point to allow for a minimum of 5 years of follow-up for risk of CLAD onset and/or death. We excluded all single lung transplants given the nature of the study, as well as heart-lung and living lobar lung transplant recipients given potential surgical confounders. We also excluded patients who did not have a 3-month VQ scan and those for whom a relative lung perfusion ratio was not reported. Individual patient consent was waived given the retrospective design and inclusion of deceased patients. The study was approved by the University of Alberta Human Research Ethics Board (Pro00095903).

Ventilation Perfusion Lung Scans

Protocolized multiplanar VQ scans were performed at 3-months post-transplant for all patients in our program prior to 2016 (this was changed to single photo emission computed tomography [SPECT] VQ scans post-2016).

A multiplanar VQ scan protocol was utilized. Our standard institutional VQ scan protocol for data analyzed in this study is as follows:

Ventilation scan: 1000 MBq (27 mCi) of ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid) nebulized and ventilated to a count rate of 1,500 counts/second. Planar images of the chest were obtained in anterior, posterior, left lateral, right lateral, anterior oblique (left and right), and posterior oblique (left and right) projections, each image to a minimum of 400,000 counts. Images were acquired using a variety of different gamma camera types with a high-resolution collimator and magnification factor of 1.46. Matrix size was 256 x 256.

Perfusion scan: 185 MBq (5 mCi) of ^{99m}Tc-MAA (macroaggregated albumin) was injected intravenously. It was ensured that the count rate was a minimum of 4 times greater than the ventilation scan (if not, more MAA was injected to titrate to this range). Planar images of the chest were obtained in anterior, posterior, left lateral, right lateral, anterior oblique (left and right), and posterior oblique (left and right) projections, each image to a minimum of 400,000 counts (same orientations as the ventilation images). Images were acquired using a variety of gamma camera types (the same camera was used for the ventilation and perfusion images in each patient) with a high-resolution collimator and magnification factor of 1.46. Matrix size was 256 x 256.

Images were typically reviewed using Pulmogam review software on Segami Oasis workstations (Columbia, Maryland).

Relative Lung Perfusion Differential

The primary risk factor was a perfusion differential on 3-months VQ scan of ≥ 10%. The differential was calculated as the absolute difference between the patient's right and left lung perfusion percentage, with the right lung perfusion percentage being higher in all cases. While no formalized guidelines exist with respect to the right-to-left differential ratio, the 55/45 distribution is most frequently recognized as the threshold of normal in the clinical setting and was remarked on as such by the interpreting clinical radiologists at our center.[9]

Outcome Definitions

The primary endpoint was time to death or retransplantation. Survival and other post-operative data were collected from our prospectively maintained program database and supporting data elements obtained from patient charts. We designated CLAD status as a persistent decline in forced expiratory volume in 1 second (FEV1) to <80% of the established baseline in the absence of other confounders as well as grade and subtype in accordance with the 2019 consensus criteria.[10] We analyzed this as time to onset of CLAD, grade 1 or higher. BLAD was defined for double lung transplant recipients as a failure to achieve both FEV1 and forced vital capacity ≥80% predicted on 2 consecutive tests at least 3 weeks apart.[8]

Statistical Analysis

We summarized continuous variables as means with standard deviations or medians with interquartile range and compared them using t-tests or Wilcoxon rank sum tests, depending on normality. Categorical variables were summarized as frequencies with percentages and compared using Fisher's exact test for binary categories and Pearson's chi-square for 3 or more categories. For ranked variables, Cochran Armitage trend testing was applied. We used Kaplan Meier estimation with log rank tests for the association between lung perfusion differential ≥10% and both survival as well as death-censored time to grade 1 CLAD onset. We also ran a logistic regression model to assess the association between unbalanced perfusion and the presence of BLAD. A *p*-value of 0.05 was considered significant. Analyses were performed on JMP 12 software (SAS Institute, Inc, Cary, NC, USA)

RESULTS

A total of 499 patients underwent transplant in our program between 2005 and 2016, 40 of whom were excluded from this study because of non-double lung transplant, 22 for death prior to 3 months, and 97 for missing data (n=64 with no VQ scan, n=33 with a VQ scan but no perfusion ratio reported) for a final cohort of 340 patients (Figure 1). Comparison of the baseline characteristics of these excluded patients with the main cohort showed minimal differences. Among the 340 patients comprising the study cohort, 169 (49%) had a relative perfusion differential of ≥10% on 3-month VQ scans. Donor and recipient baseline characteristics were similar when compared with their counterparts with normal perfusion differential (table 1). Notably, perfusion differential was not related to

donor-recipient matching parameters such as height or sex. The unbalanced perfusion group had longer total hospital stays (24 [19-43] days vs. 21 [16-32] days; *p*=0.004) and lower peak post-transplant FEV1% predicted (90% vs. 96%; *p*=0.006).

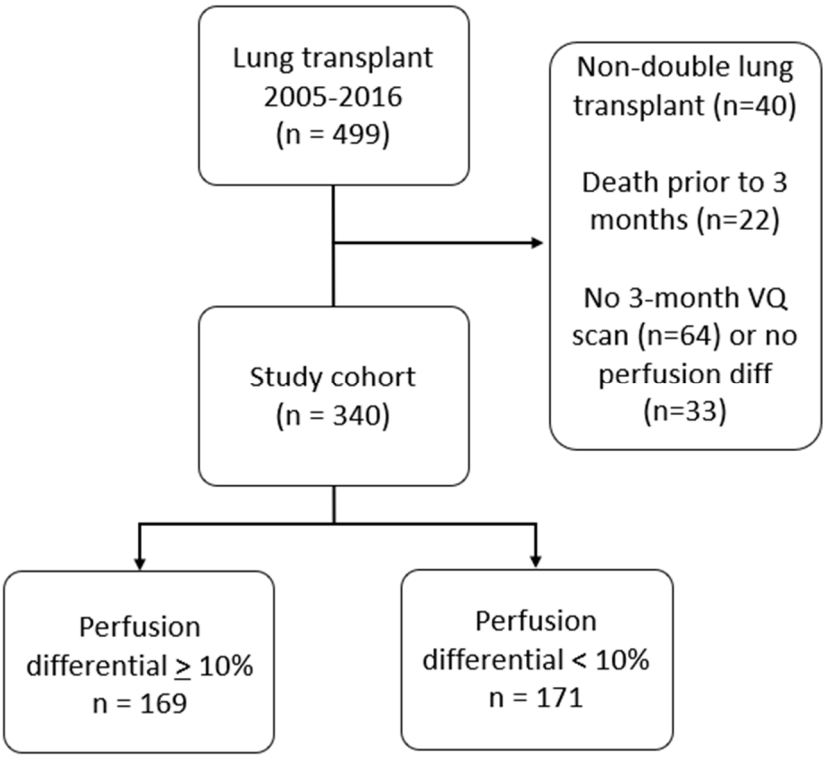

Figure 1. Study cohort.

Ventilation perfusion scans

The median right-left perfusion differential was 52 [49-56] vs. 48 [44-51]), performed at a median of 85 days (IQR 74-91) post-transplant (table 1). In patients where both ventilation and perfusion differentials were reported (n=291), we tested the association between the two measurements. Differential ventilation was highly related to differential perfusion, both via correlation when analyzed continuously (correlation coefficient 0.5397, p<0.0001; Figure 2) and via agreement statistic when classified as > or ≤ 10% (Cohen's kappa = 0.5196, p<0.0001; supplementary figure 1). The R^2 associated with ventilation

differential as a determinant of perfusion differential was 0.2913, indicating that while ventilation plays a role in determining perfusion, other factors account for most of the variation in the perfusion differentials.

We also quantified the magnitude of the percentage of the ventilation-perfusion mismatch for this cohort (table 1). Patients with an increased perfusion differential were more likely to have quantifiable VQ mismatch as continuous measurements (correlation coefficient 0.174, p=0.0029) but not when analyzed via thresholds of $\leq$ or > 10% (p=0.205).

A small number of patients (n=27) had mismatched perfusion defects [diagnosed by the interpreting radiologist as intermediate or high risk for pulmonary embolism] on their routine 3-month VQ scan.[11] These diagnoses were not associated with differences in the perfusion differential (p=0.9369) or the ventilation differential (p=0.0920).

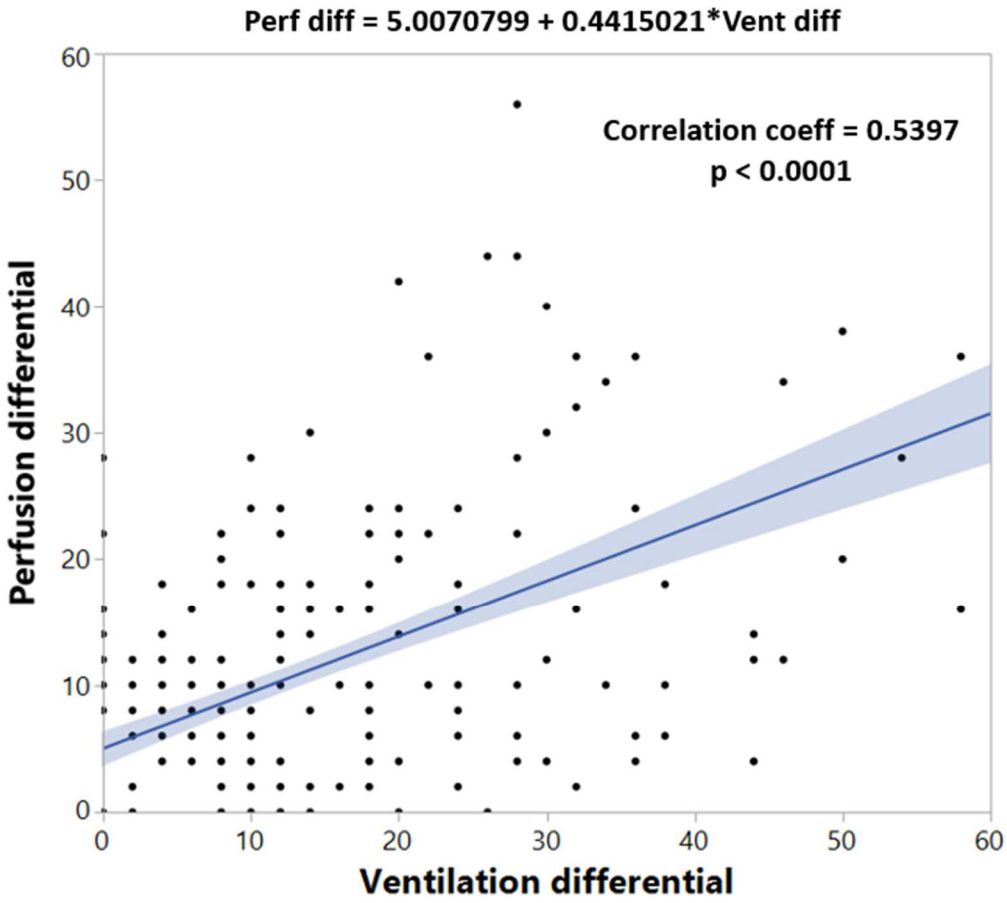

Figure 2. Perfusion differential as a function of ventilation differential

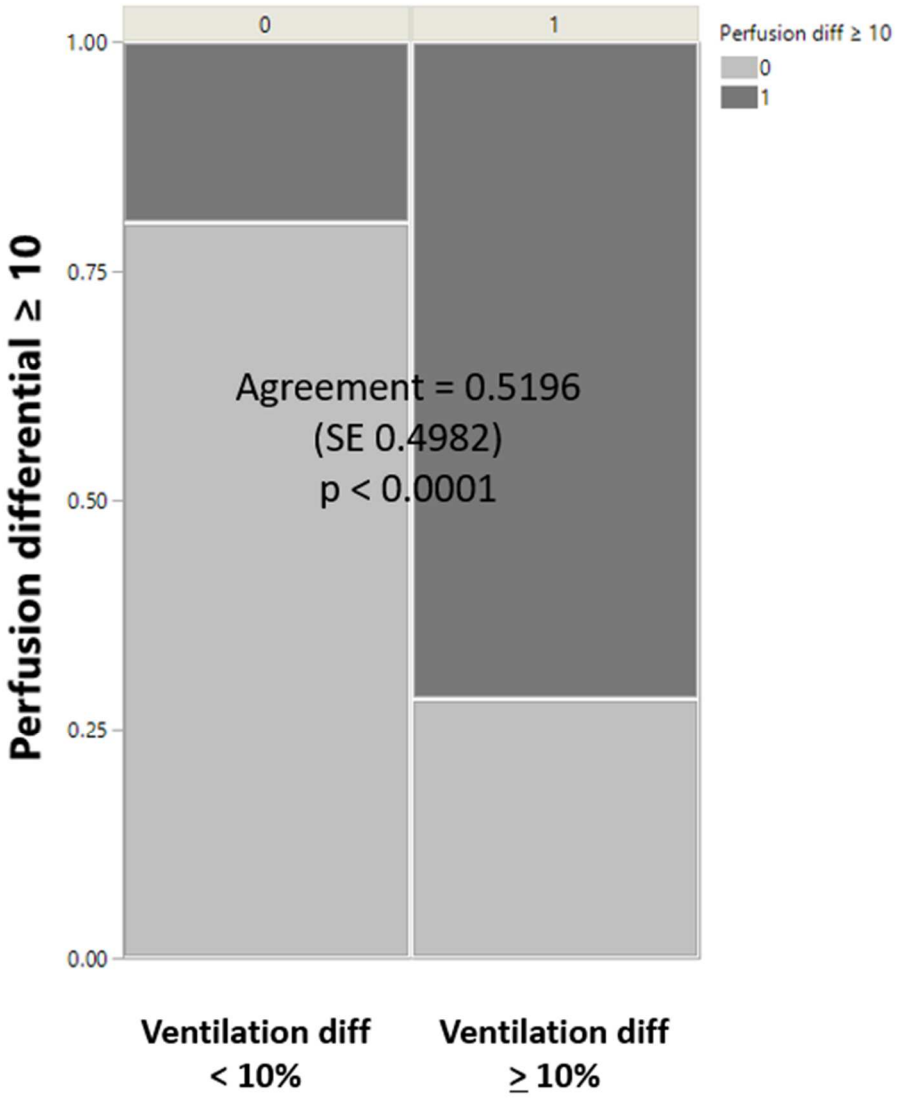

Figure 3. Relationship between perfusion differential and ventilation differential.

Table 1. Patient characteristics and outcomes stratified by perfusion difference ≥ 10%

Characteristic	Overall (n=340)	Perfusion difference ≥ 10% (n=169)	Perfusion difference < 10% (n=171)	p-value
Age in years, mean	52 (13)	52 (14)	53 (13)	0.630
Female sex	116 (34)	66 (39)	50 (29)	0.067
BMI, mean	24.8 (5.0)	25.2 (5.2)	24.4 (4.8)	0.131
Diagnosis				0.275
Bronchiectasis	48 (14)	25 (15)	23 (13)	
Interstitial lung disease	137 (40)	70 (41)	67 (39)	
Obstructive lung disease	137 (40)	63 (37)	74 (43)	
Pulmonary vascular disease	10 (3)	8 (5)	2 (1)	
Other	8 (2)	3 (2)	5 (3)	
Recipient bridging				0.313
Ventilated	21 (6)	8 (5)	13 (8)	
ECMO	14 (4)	9 (5)	5 (3)	
None	305 (90)	152 (90)	153 (89)	
CMV mismatch	68 (20)	35 (21)	33 (19)	0.787
Cross match positive, n=321	50 (16)	30 (19)	20 (12)	0.124
Intraoperative support				0.250
CPB	282 (83)	135 (80)	147 (86)	
ECMO	28 (8)	15 (9)	13 (8)	
None	30 (9)	19 (11)	11 (6)	
Induction therapy				0.272
IL-2 receptor antagonists	190 (56)	101 (60)	89 (52)	
Anti-lymphocyte antibody	141 (41)	65 (38)	76 (44)	

None	9 (3)	3 (2)	6 (4)	
Maintenance therapy				
Tacrolimus	324 (95)	162 (96)	162 (95)	0.799
MMF	335 (99)	165 (98)	170 (99)	0.368
Donor				
Age in years, mean	39 (17)	40 (18)	38 (16)	0.299
Female sex	206 (46)	163 (47)	43 (43)	0.663
BMI, mean	25.6 (5.1)	25.5 (4.9)	25.6 (5.3)	0.826
Smoking > 20 pack years, n=313	44 (14)	24 (15)	20 (13)	0.520
Ischemic time in minutes, mean	351 (120)	355 (126)	346 (115)	0.489
Donor race				0.313
Caucasian	251 (74)	124 (73)	127 (74)	
Black	5 (1)	3 (2)	2 (1)	
Asian	19 (6)	13 (8)	6 (4)	
Other	65 (19)	29 (17)	36 (21)	
Donor-recipient height diff (cm)		-0.3 (6.2)	0.3 (6.8)	0.370
Donor-recipient height ratio		1.00 (0.04)	1.00 (0.04)	0.370
Donor-recipient sex match				0.214
Male-male	158 (46)	72 (43)	86 (50)	
Male-female	28 (8)	18 (11)	10 (6)	
Female-male	66 (19)	31 (18)	35 (20)	
Female-female	88 (26)	48 (28)	40 (23)	
Post-operative outcomes				
Intubation time in hours, median	61 (32-156)	66 (33-192)	57 (32-123)	0.193
Hospital LOS in days, median	23 (18-37)	24 (19-43)	21 (16-32)	**0.004***
Grade 3 PGD at 48 or 72h	53 (16)	25 (15)	28 (16)	0.765
BLAD	126 (37)	72 (42)	54 (32)	**0.043***

CLAD	97 (29)	55 (33)	42 (25)	0.119
Time to CLAD in days, mean	1378 (940)	1263 (888)	1528 (997)	0.177
Max FEV1 % predicted, mean	93 (20)	90 (21)	96 (19)	**0.006***
1-year survival	334 (98)	167 (99)	167 (98)	0.685
Follow-up duration, median	1789 (1170-2951)	1568 (1028-2751)	2081 (1393-3140)	**0.001***
Ventilation perfusion scans				
Time to VQ scan, days	85 (74-91)	85 (73-91)	85 (75-91)	**0.909**
Ventilation differential	10 (4-18)	14 (10-24)	6 (2-10)	**<0.001**
Ventilation differential > 10%, n=291	148 (50%)	106 (79)	42 (27)	**<0.001**
Perfusion differential	8 (4-16)	16 (12-22)	4 (0-8)	**<0.001**
VQ mismatch %, n=291	1 (0-6)	3 (0-7)	0 (0-5)	**0.002**
VQ mismatch > 10%, n=291	35 (12)	20 (15)	15 (10)	0.206

Means are presented with standard deviations, medians with interquartile range and counts with percentages. BMI = body mass index in kg/m2; CMV = cytomegalovirus; CPB = cardiopulmonary bypass; ECMO = extracorporeal membrane oxygenation; LOS = length of stay; PGD = primary graft dysfunction; BLAD = baseline lung allograft dysfunction; CLAD = chronic lung allograft dysfunction; FEV1 = forced expiratory volume in 1 second; VQ = ventilation perfusion

Relationship to other diagnostic modalities

85 patients (25%) had a significant identifiable abnormality on radiographic assessment via computed tomography, chest x-ray or bronchoscopic assessment. These abnormalities were more common in patients with a wide perfusion differential (38% vs. 12%, p<0.001). A summary of findings is depicted in table 2, the most common of which were: diaphragmatic hemiparesis or lung size asymmetry; asymmetric pleural effusion; and subclinical pulmonary embolism or other vascular occlusions.

Table 2. Identifiable radiographic or endoscopic abnormalities stratified by wide perfusion differential.

Characteristic	Overall (n=340)	Perfusion difference ≥ 10% (n=169)	Perfusion difference < 10% (n=171)	p-value
Any identifiable abnormality	85 (25)	64 (38)	21 (12)	<0.001
Vascular occlusion	29 (9)	15 (9)	14 (8)	
Pleural effusion	18 (5)	15 (9)	3(2)	
Lung size asymmetry	16 (5)	15 (9)	1 (1)	
Bronchial stenosis	7 (2)	6 (4)	1 (1)	
Atelectasis/collapse	6 (2)	6 (4)	0 (0)	
Parenchymal changes or infection	5 (2)	3 (2)	2 (1)	
Pneumothorax	3 (1)	3 (2)	0 (0)	
Air trapping	1 (<1)	1 (1)	0 (0)	

Means are presented with standard deviations, medians with interquartile range and counts with percentages.

Perfusion differential and overall survival

There were 119 deaths and 1 retransplant over the study duration, and as such we referred to the primary outcome as overall survival. Unadjusted Kaplan-Meier survival analysis showed that patients with perfusion differential ≥10% had poorer overall survival (Figure 3, p=0.011). 1-year survival was similar between the groups. When the analysis is conducted stratifying the population by the presence of an identifiable abnormality on imaging or endoscopy, the association between wide perfusion differential and poorer overall survival persists in the 225 patients with no identifiable abnormality (p=0.036) but not in the 85 patients with an associated identifiable abnormality (p=0.774).

Perfusion differential remained associated with poorer overall survival in an adjusted proportional hazards model accounting for age, sex, pulmonary diagnosis, and an identifiable radiographic/endoscopic abnormality (HR 1.48 [95% CI 1.02-2.18]; p=0.041).

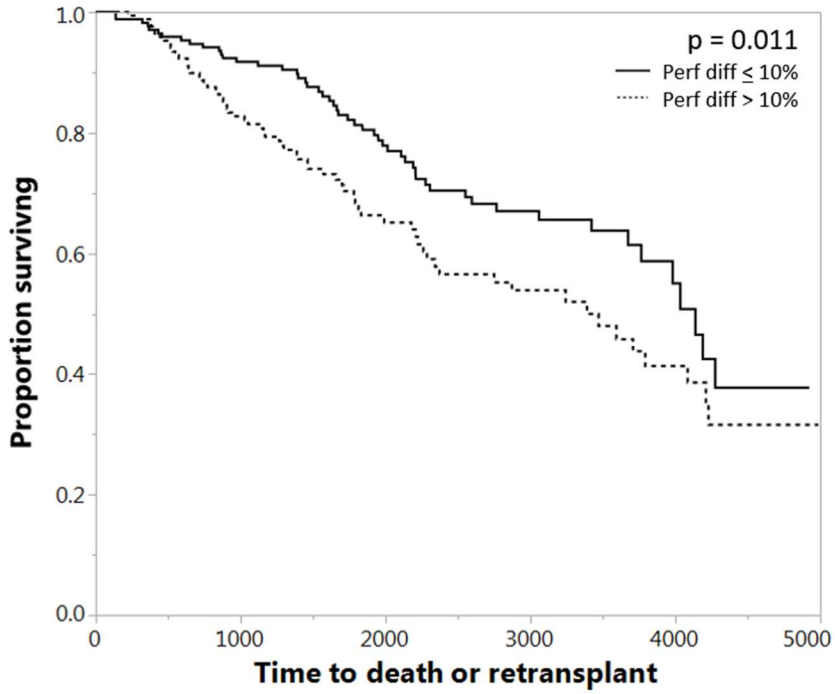

Figure 4. Time to death or retransplant stratified by perfusion differential >10% (solid line) or ≤ 10% (dotted line)

Perfusion differential and lung function at time of 3-month VQ

A wide perfusion differential was associated with lower lung function at the time of VQ assessment on 3-month post-transplant spirometry in terms of both the forced expiratory volume in 1 second (FEV1) percent predicted (65% vs. 71%, p<0.001) and the forced vital capacity percent predicted (64% vs. 69%, p=0.002). The relationship between perfusion differential and 3-month FEV1 in liters was stronger than that between ventilation differential and 3-month FEV1 (Figure 4). Wide perfusion differential remained associated with a lower 3-month FEV1 even after adjusting for an identifiable abnormality on imaging/endoscopy in a linear regression model (B = -0.011 [standard error 0.004], p=0.007].

Wide perfusion differential was not simply a reflection of low spirometry, however. Patients with a 3-month FEV1< 80% were more likely to have a wide perfusion differential but were not consistently identified by this threshold (k 0.148, p=0.002), nor by a wide ventilation differential > 10% (k 0.105, p=0.040). Furthermore, a logistic model of 3-month FEV1 < or $\underline{\geq}$ 80% as a function of a perfusion differential as a continuous predictor was associated with an area-under-the-curve of only 0.578. Of note, a perfusion differential of 10 was selected by the model as the optimal cut point.

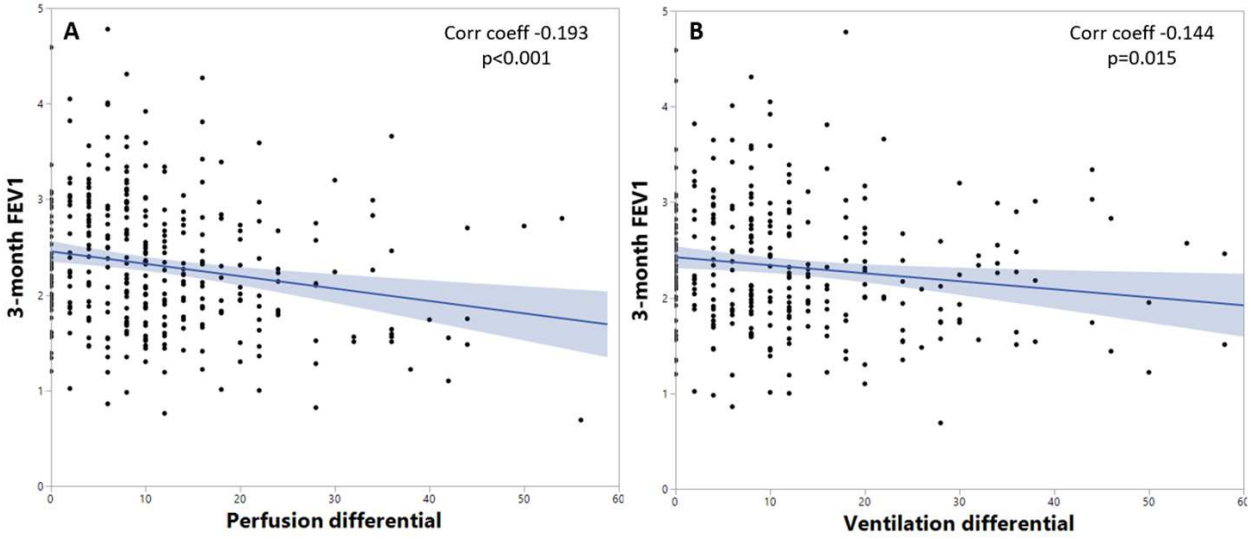

Figure 5. 3-month FEV1 in liters as a function of (A) perfusion differential and (B) ventilation differential

Perfusion differential and the future risk of chronic lung allograft dysfunction onset

CLAD onset of at least grade 1 occurred in 97 patients over the study period. Perfusion differential ≥ 10% was associated with earlier death-censored time to CLAD onset in unadjusted Kaplan-Meier analysis (Figure 5, p=0.012). Wide perfusion differential remained significantly associated with an earlier death-censored time to CLAD onset in a bivariate proportional hazards model adjusting for the presence of an identifiable radiographic or endoscopic abnormality alone (HR 1.57 [95% CI 1.04 – 2.40]; p=0.047) as well as in a multivariable model adjusting for the presence of an identifiable abnormality as well as variables known to be associated with increased CLAD risk and those identified in this population [induction therapy, cytomegalovirus mismatch, use of prophylactic azithromycin, human leukocyte antibody flow cytometric crossmatch positive at transplant, and intraoperative support modality] (HR 1.61 [95% CI 1.05 – 2.48]; p=0.028).

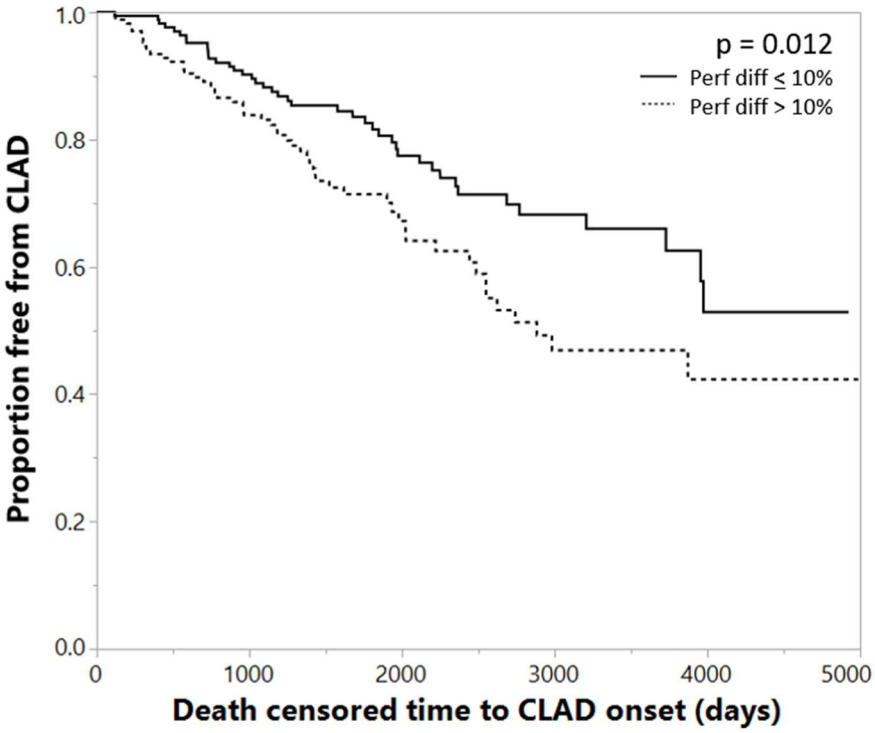

Figure 6. Death censored time to onset of CLAD stratified by perfusion differential >10% (solid line) or ≤10% (dotted line).

Perfusion differential and the future risk of baseline lung allograft dysfunction

BLAD was present in 126 patients. Perfusion differential increased with increasing BLAD grade (Figure 6A), as did ventilation differential (6B). Perfusion differential > 10% was associated with an increased risk of post-transplant BLAD (odds ratio 1.61 [95% CI 1.03-2.51]; *p*=0.036). Perfusion differential > 10% was however no longer associated with BLAD risk after adjustment for the presence of an identifiable radiographic or endoscopic abnormality alone (OR 1.34 [95% CI 0.84 – 2.14; p=0.222]) or adjusted for these identifiable abnormalities as well as previously documented BLAD risk factors and factors associated with BLAD in this population including obesity [BMI > 30], pulmonary diagnosis, donor age and heavy donor smoking history (OR 1.11 [95% CI 0.65 – 1.89];

p=0.690). Of note, the presence of an identifiable radiographic abnormality remained strongly associated with BLAD in this model.

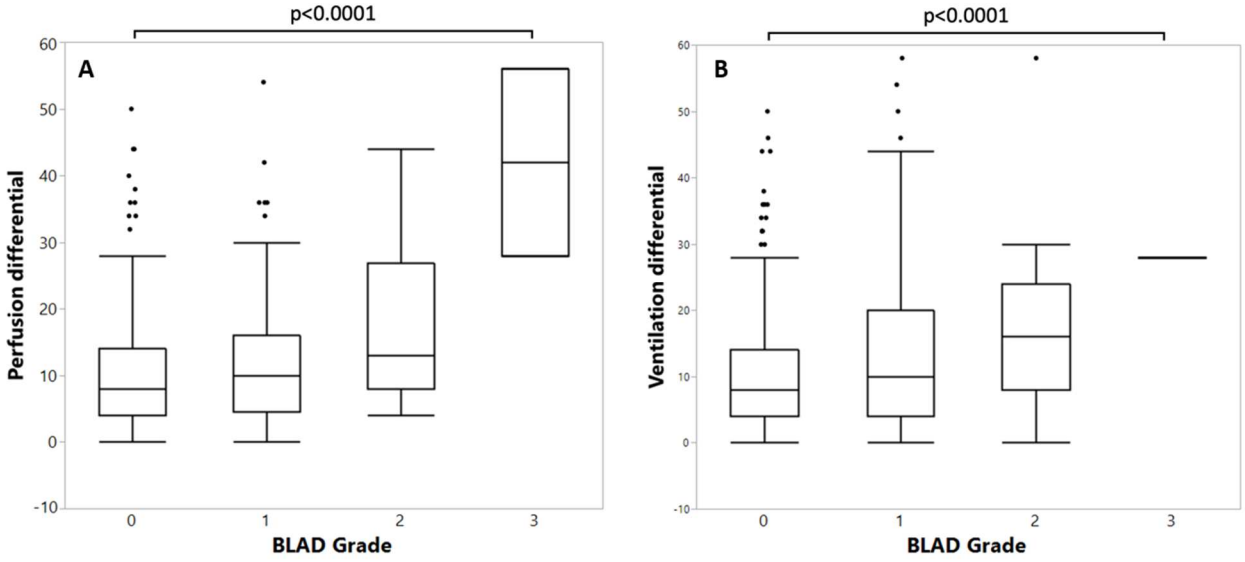

Figure 7. Perfusion differential (A) and ventilation differential (B) by BLAD grade

DISCUSSION

An abnormally wide lung perfusion differential at 3-months post-transplant was common in our cohort of lung transplant recipients and associated with an increased adjusted risk of death, lower lung function at time of scan and of future adjusted risk of chronic lung allograft dysfunction onset.

The purpose of this study was to characterize the population perfusion differential in lung transplants in the early post-operative course and its utility as a marker of future risk. The first objective was met with some surprising findings. We anticipated perfusion differential would most likely reflect the ventilation differential, as hypoxic vasoconstriction indicates that perfusion is, at least to a degree, dependent on ventilation. While there was a

demonstrable relationship, it was weaker than expected and other factors appear to be modulating the perfusion differential. These could potentially be surgical in nature, either technical aspects or the differential integrity of the vascular anastomoses. Given there are no observable associations with donor or donor-recipient matching parameters, it seems unlikely these are purely donor-associated. Most importantly, it does not in our population appear to be being driven by early post-transplant complications such as primary graft dysfunction and prolonged ventilation. Although a substantial number of patients with a wide perfusion differential had a potentially localizing radiographic or endoscopic abnormality, the majority did not. This leads us to speculate that wide perfusion differential reflects intrinsic abnormalities of the large or small vessels of the pulmonary vasculature (or potentially the parenchyma) which is either predominantly unilateral or differentially affects both lungs and not otherwise detectable via imaging.

The observed association between a wide perfusion differential and reduced survival in our study could occur through several mechanisms. First, this could reflect an early marker of CLAD physiology and our results could be in keeping with this. While CLAD by definition cannot be diagnosed in the first 3-months post-transplant, this does not necessarily preclude the emergence of early markers of risk.[12–14] We speculate that perfusion differential could represent matched defects unevenly distributed across the right and left lungs, a marker of heterogeneity which manifests prior to frank radiographic findings such as air-trapping or overt physiologic deterioration [though lung function was notably lower in these patients at time of assessment].[15,16] VQ scans have been shown to detect air trapping at an earlier timepoint than expiratory CT scans, and the presence

of this finding has in turn been associated with subsequent development of CLAD.[17] However, it is unlikely that these changes are purely secondary to ventilatory changes and hypoxic vasoconstriction given the relatively weak relationships between the two that we observed. Previous MRI-based studies have demonstrated that the emergence of perfusion abnormalities occurred prior to deterioration in spirometry in lung transplant recipients who went on to develop CLAD, and perfusion differential may be reflecting similar information.[18] Furthermore, although a subset of the wide perfusion differentials appear to be explainable by observable abnormalities on other diagnostic modalities, the majority (62%) of this cohort had no associated identifiable findings. More importantly, the prognostic association of wide perfusion differential with survival and CLAD remained after adjustment for these abnormalities. Finally, we do not suspect that these are related to significant pulmonary vascular occlusions or emboli and the related mortality risk.[19]

The lack of adjusted association between wide perfusion differential and BLAD is surprising and contrary to our expectations, particularly given these patients appear to have lower lung function at the outset. It is possible our results represent a false negative but taken at face value, there appears to be no relationship after accounting for other factors. It is notable that BLAD patients more frequently had identifiable radiographic or endoscopic abnormalities, which aligns with prior speculation that BLAD does not reflect a single pathophysiology but rather a physiologic presentation with a variety of etiologies.[8] It is possible then based on the presented data that a subset of wide perfusion differentials reflect other pathologies which may give rise to BLAD physiology, but that the majority of wide perfusion differentials seem to exert their influence on CLAD and increased risk of

death independently of baseline dysfunction. Given our prior work, we speculate this could be because BLAD manifests more in the airways (indeed this is how it is defined) while perfusion differential is a more vascular phenomenon and perhaps reflects a distinct entity.[20]

Our study has limitations. First, it is important to note that our main outcome reflects a form of conditional survival, as patients who died prior to 3-months post-transplant did not undergo VQ scans and were excluded. Second, VQ scans were not protocolized in our program prior to 2008 and thus patients transplanted from 2004-2007 represent a significant portion of our missing data; 28 of 64 of patients who did not receive VQ scans were transplanted during that period. Third, VQ scans were not routinely performed at follow-ups beyond 3-months post-transplant, so we are unable to comment on the subsequent evolution of these changes or the influence of change over time on survival. Finally, the normal threshold for right-left lung relative perfusion of 55-45 is accepted but generally based on consensus alone. However, we note that our logistic model of 3-month FEV1 $\geq$ or <80% identified 10% as the optimal cut point. As well, a previous study that quantified split perfusion on VQ scans of 206 healthy patients found the mean right-to-left lung perfusion ratio to be 52.5%/47.5%, and this is very similar to the mean ratio calculated for our series of 52.6%/47.4%.[9] They additionally determined that the population variance in their study was quite small and that 55-45 was more than one standard deviation away from the mean for their series. Taking these previous findings as well as the summary of our data into account, we feel the commonly used 55-45 differential is a reasonable threshold for normal. There are also conceivably situations

where the 10% differential threshold would miss deviations from a normal split, namely when the physiological split is reversed with left lung perfusion being greater than right lung perfusion but the absolute differential still being within 10%. However, this type of misclassification would bias results toward the null and produce a false negative rather than a false positive.

A wide lung perfusion differential on routine post-transplant VQ scan was common in our cohort – accounting for half of the cases - and associated with an increased risk of death, lower lung function at time of assessment and with future risk of CLAD. This metric may serve as a novel early marker of risk for poor post-transplant outcomes as well as provide additional insight into early CLAD physiology and outlining a role for further investigation into the underlying mechanisms.

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CHAPTER 3 – Mismatched Perfusion Defects on Routine Ventilation-Perfusion Scans after Lung Transplantation

INTRODUCTION

Pulmonary emboli (PE) detected incidentally on imaging studies is a clinical entity with unclear prognostic and treatment implications and current guidelines continue to reflect the limited evidence base for treatment with therapeutic anticoagulation versus conservative management.[1] Mismatched perfusion defects (PD) on routine studies in the post-transplant setting are a unique subset of this diagnostic challenge, as the radiographic changes may represent donor- or recipient-derived thrombus, non-thrombotic material or sequelae of surgical manipulation of the pulmonary vasculature.[2] Pulmonary embolism has been shown to occur commonly in mechanically ventilated patients, a patient population that provides the source for most lung donors.[3] In many of these cases, these changes would not be reflective of recipient hypercoagulability or predictive of future PE risk and calls into question whether routine anticoagulation is required. The long-term implications of early PE in the transplanted lungs are also poorly understood. Most studies on the subject have focused on recipients who have received donor lungs identified to have clots on the pre-transplant exploratory flush; these have yielded variable results with respect to effect on survival and lung function.[4–7] In the post-transplant setting, symptomatic PE has been shown to be associated with a worse prognosis, as would be expected in the non-transplant setting.[8,9]

Our program performs routine ventilation-perfusion (VQ) scans at 3-months post-transplant for all recipients to establish baseline airway and vascular function. VQ scans are sensitive in the detection of PE, and these routine studies sometimes demonstrate mismatched perfusion defects (perfusion defects with normal ventilation) interpreted as suspicious for PE.[10–12] Our objective was to test whether there was an association with patients with these mismatched perfusion defects and 1-year survival as well as overall survival, both treated and untreated with anticoagulation. We also sought to evaluate the association with long-term lung function in terms of the risk of chronic lung allograft dysfunction (CLAD) onset and baseline lung allograft dysfunction (BLAD). We hypothesized that perfusion defects in this context would carry a benign prognosis for post-transplant survival and long-term lung function.

MATERIALS AND METHODS

Population

We reviewed all adult patients who underwent double lung transplant at the University of Alberta hospital between January 1, 2005 and December 31, 2016 with available 3-months post-transplant VQ scans. We excluded all single lung, heart-lung, and living lobar lung transplant recipients. Patient consent was waived based on the retrospective design and the inclusion of deceased patients. This study was approved by the institutional review board (Pro00095903).

Lung procurement

Donor lungs at our center are procured conventionally. Systemic anticoagulation is achieved with 40,000 units of Heparin intravenously 10 minutes prior to cross-clamping the ascending aorta. Lung specific preservation is achieved with direct injection of 1000 micrograms of Prostaglandin (PGE1) into the main pulmonary artery and subsequent pulmonary arterial cannulation using an anterograde flush of 4L of pulmonoplegic solution (Perfadex, XVIVO Perfusion, Sweden) by gravity and venting through the left atrial appendage. After donor cardiectomy, a retrograde flush is performed with 250 mL per pulmonary vein (total of 1L) with drainage through the pulmonary artery.

Post-transplant VTE prevention

Post-transplant venous thromboembolism (VTE) prophylaxis in the immediate post-operative period consists of sequential compressive devices on the lower extremities, followed by the institution of low molecular heparin (LMWH) administered subcutaneously daily (tinzaparin) if there is adequate hemostasis in the first 24-48 hours. Compression is withdrawn once the patient is ambulatory, but patients remain on LMWH for the duration of their hospital stay, which averages 3-4 weeks.

Ventilation perfusion lung scans and perfusion defects

Protocolized multiplanar VQ scans were performed at 3-months post-transplant for all patients in our program prior to 2016 (this was changed to single photo emission computed tomography [SPECT] VQ scans post-2016). The risk group was patients whose VQ studies showed mismatched segmental perfusion defects judged to be high or intermediate probability for PE (ie. a minimum of one >25% segment mismatched

perfusion defect) by the interpreting radiologist using the modified PIOPED II (Prospective Imaging of Pulmonary Embolism Diagnosis) criteria. This corresponds to a post-test probability of 95% and 48% respectively in patients with a high pre-test clinical probability and a 36% or higher chance of PE irrespective of clinical probability.[13,14] We referred to these as mismatched perfusion defects given uncertainty about whether they reflected pulmonary emboli.

A multiplanar VQ scan protocol was utilized. Our standard institutional VQ scan protocol for data analyzed in this study is as follows:

Ventilation scan: 1000 MBq (27 mCi) of ^{99m}Tc-DTPA (diethylenetriaminepentaacetic acid) nebulized and ventilated to a count rate of 1,500 counts/second. Planar images of the chest were obtained in anterior, posterior, left lateral, right lateral, anterior oblique (left and right), and posterior oblique (left and right) projections, each image to a minimum of 400,000 counts. Images were acquired using a variety of different gamma camera types with a high-resolution collimator and magnification factor of 1.46. Matrix size was 256 x 256.

Perfusion scan: 185 MBq (5 mCi) of ^{99m}Tc-MAA (macroaggregated albumin) was injected intravenously. It was ensured that the count rate was a minimum of 4 times greater than the ventilation scan (if not, more MAA was injected to titrate to this range). Planar images of the chest were obtained in anterior, posterior, left lateral, right lateral, anterior oblique (left and right), and posterior oblique (left and right) projections, each image to a minimum

of 400,000 counts (same orientations as the ventilation images). Images were acquired using a variety of gamma camera types (the same camera was used for the ventilation and perfusion images in each patient) with a high-resolution collimator and magnification factor of 1.46. Matrix size was 256 x 256.

Images were typically reviewed using Pulmogam review software on Segami Oasis workstations (Columbia, Maryland).

Endpoints

The primary endpoint was 1-year retransplant free survival. Secondary endpoints were time to death or retransplantation, time to CLAD onset and BLAD status. CLAD status, grade, and subtype were designated in accordance with the 2019 International Society for Heart and Lung Transplantation consensus criteria after ruling out non-CLAD confounders.[15] The associated endpoint was time to CLAD onset of grade 1 or higher. We defined BLAD according to our previously published definition as a failure to achieve both forced expiratory volume in 1 second (FEV1) and forced vital capacity of at least 80% predicted on 2 consecutive spirometry at least 3 weeks apart after double lung transplantation.[16] Data was obtained from our prospectively maintained program database with additional supporting data from patient charts.

Statistical analysis

Continuous variables were presented as means with standard deviations or medians with interquartile ranges (IQR) and compared using *t*-tests or Wilcoxon rank sum tests

depending on distribution. Categorical variables were expressed as frequencies and percentages and compared with Fisher's exact tests for binary categories and Pearson's chi-square tests for >2 categories. We used chi-testing to evaluate the association between PD status and 1-year survival, Kaplan Meier estimation with log rank tests for both retransplant-free survival as well death-censored time to grade 1 CLAD onset, and logistic regression to assess the relationship for the occurrence of BLAD. We conducted all analyses on JMP 12 software (SAS Institute, Inc, Cary, NC, USA) and considered a two-sided p-value of 0.05 statistically significant.

RESULTS

Baseline characteristics

A total of 499 patients received lung transplants in our program between 2005 and 2016. We excluded 40 patients based on receiving a non-double lung transplant to facilitate designating normal versus abnormal baseline function, 22 for death prior to 3 months, and 64 for no retrievable VQ scan (Figure 1). The remaining 373 patients who met inclusion criteria were not clinically suspected to have PE at their 3-month follow-up, but among them 35 patients (9%) had VQ scans with perfusion defects interpreted as high or intermediate probability for PE. Baseline characteristics were similar between PD and no PD groups, including all studied donor variables (Table 1).

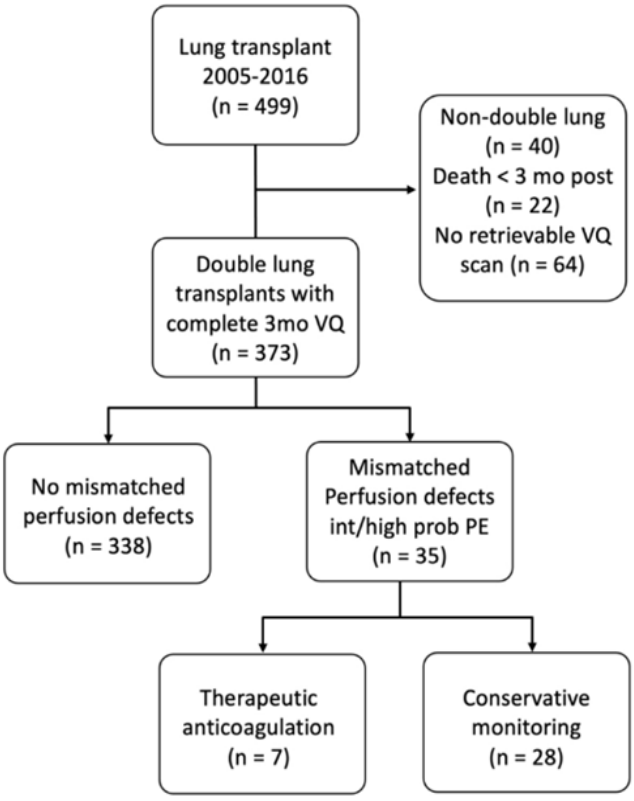

Figure 1. Study cohort

Table 1. Baseline characteristics stratified by mismatched perfusion defect on 3-months VQ scan

Characteristic	Overall (n=373)	Perfusion defect (n=35)	No perfusion defect (n=338)	p-value
Age in years, mean	52 (13)	52 (14)	52 (13)	0.732
Female sex	127 (34)	15 (43)	112 (33)	0.264
BMI, mean	24.9 (5.0)	24.6 (5.2)	24.9 (5.0)	0.713
Diagnosis				0.309
Bronchiectasis	52 (14)	8 (23)	44 (13)	
Interstitial lung disease	150 (40)	13 (37)	137 (41)	
Obstructive lung disease	150 (40)	11 (31)	139 (41)	
Pulmonary vascular disease	12 (3)	1 (3)	11 (3)	
Other	9 (2)	2 (6)	7 (2)	
Recipient bridging				0.468
Ventilated	22 (6)	2 (6)	20 (6)	
ECMO	14 (4)	0 (0)	14 (4)	
None	337 (90)	33 (94)	304 (90)	
CMV mismatch	77 (21)	9 (25)	68 (20)	0.510
Intraoperative support				0.579
CPB	311 (83)	29 (83)	282 (83)	
ECMO	29 (8)	4 (11)	25 (7)	
None	33 (9)	2 (6)	31 (9)	
Induction therapy				0.531
IL-2 receptor antagonists	205 (55)	21 (60)	184 (54)	

Anti-lymphocyte antibody	158 (42)	14 (40)	144 (43)	
None	10 (3)	0 (0)	10 (3)	
Maintenance therapy				
Azathioprine	10 (3)	3 (9)	7 (2)	0.058
Cyclosporine	59 (16)	4 (11)	55 (16)	0.627
MMF	368 (99)	35 (100)	333 (99)	1.000
Tacrolimus	350 (94)	34 (97)	316 (93)	0.710
Donor				
Age in years, mean	39 (16)	39 (15)	39 (17)	0.940
Female sex	171 (46)	17 (49)	154 (46)	0.859
BMI, mean	25.5 (5.3)	26.2 (6.1)	25.5 (5.2)	0.518
Smoking > 20 pack years	47 (14) (n=344)	3 (9) (n=32)	44 (14) (n=312)	0.595
Ischemic time in minutes, mean	350 (119)	349 (98)	350 (120)	0.964
Donor type				1.000
DCD	13 (3)	1 (3)	12 (4)	
NDD	360 (97)	34 (97)	326 (96)	
Donor race				0.643
Caucasian	275 (74)	27 (77)	248 (73)	
Black	6 (2)	0 (0)	6 (2)	
Asian	20 (5)	3 (9)	17 (5)	
Other	72 (19)	5 (14)	67 (20)	

BMI = body mass index in kg/m2; CMV = cytomegalovirus; CPB = cardiopulmonary bypass; ECMO = extracorporeal membrane oxygenation; DCD = donation after cardiac death; NDD = neurological determination of death

Ventilation perfusion scan characteristics

The extent of the mismatched perfusion area associated with suspected pulmonary embolism on the VQ scans were generally deemed medium or large size and were most commonly a single segmental defect (Table 2). The distribution of perfusion defects showed a lower lobe predominance (Figure 2).

Table 2. Extent and number of mismatched perfusion defects on 3-months VQ scan

VQ scan characteristics	Perfusion defect (n=35)
Perfusion defect extent, n (%)	
Small	5 (14)
Medium	14 (40)
Large	16 (46)
Number of perfusion defects present, n (%)	
1	20 (57)
2	8 (23)
3	6 (17)
4	1 (3)

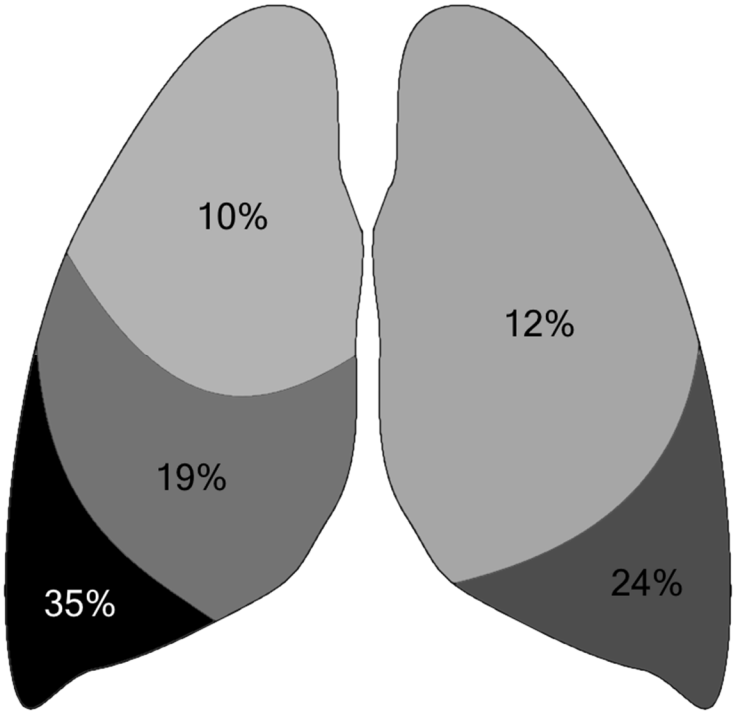

Figure 2. Lobar distribution of mismatched perfusion defects in patients with mismatched perfusion defects on 3-months VQ scan

Therapeutic anticoagulation

Of the 35 patients with perfusion defects, 7 (20%) received therapeutic anticoagulation (low molecular weight heparin bridging to warfarin or direct oral anticoagulant) for presumed PE and the remainder were managed conservatively. In the treated group, anticoagulation was initiated for one patient with Factor 5 Leiden thrombophilia and the other 6 patients had positive findings for deep vein thrombosis (DVT) on lower extremity venous ultrasound. 5 patients were treated with short courses ranging from 44 to 190 days, and two patients with long-term anticoagulation.

None of the 35 patients with PD developed subsequent symptomatic PE during their post-transplant course. 6 of the PD patients had a follow up VQ scan at least 3-months later, and all still had mismatched perfusion defects at the original locations (ie. chronic perfusion defects).

Primary graft dysfunction and post-operative outcomes

Patients with PD were less likely to have had severe primary graft dysfunction (3% vs. 19%; p=0.03). Duration of mechanical ventilation, intensive care unit and hospital length of stay were all similar between PD and No PD groups (Table 3).

Table 3. Post-operative and long-term outcomes stratified by perfusion defect on 3-months VQ scan

Characteristic	Overall (n=373)	Perfusion defect (n=35)	No perfusion defect (n=338)	p-value
Intubation time in hours, median	63 (33-159)	45 (25-102)	67 (33-178)	0.063
Hospital LOS in days, median	27 (19-45)	26 (16-41)	27 (19-45)	0.400
Grade 3 PGD at 48 or 72 hours	64 (17)	1 (3)	63 (19)	**0.027***
Baseline lung allograft dysfunction	144 (39)	14 (40)	130 (38)	0.857
Grade 1	110 (29)	13 (37)	97 (29)	
Grade 2	31 (8)	1 (3)	30 (9)	
Grade 3	3 (0)	0 (0)	3 (1)	
Chronic lung allograft dysfunction	106 (28)	11 (31)	95 (28)	0.700

Grade 1		8 (23)	40 (12)	
Grade 2		1 (3)	21 (6)	
Grade 3		0 (0)	16 (5)	
Grade 4		2 (6)	18 (5)	
Max FEV1 % predicted, mean	93 (21)	94 (20)	92 (21)	0.584
1-year survival	366 (98)	35 (100)	331 (98)	1.000

Survival

There were 134 deaths and 1 retransplant over a median follow-up duration of 1814 days (interquartile range 1164-2980). As such we referred to the primary outcome as overall survival. There were 14 deaths within the PD group over the study period and none were attributable to sudden cardiac arrest. Patients with PD had similar 1-year survival (100% vs. 98%, p=1.000) and overall survival (Figure 3) compared with patients without PD. As well, neither 1-year nor overall survival differed by therapeutic anticoagulation status (No PD 98%, PD with anticoagulation 100%, PD with no anticoagulation 100%). The high 1-year survival in the overall cohort is attributable to censoring patients who were unable to undergo a 3-month VQ study, and as such reflects conditional survival.

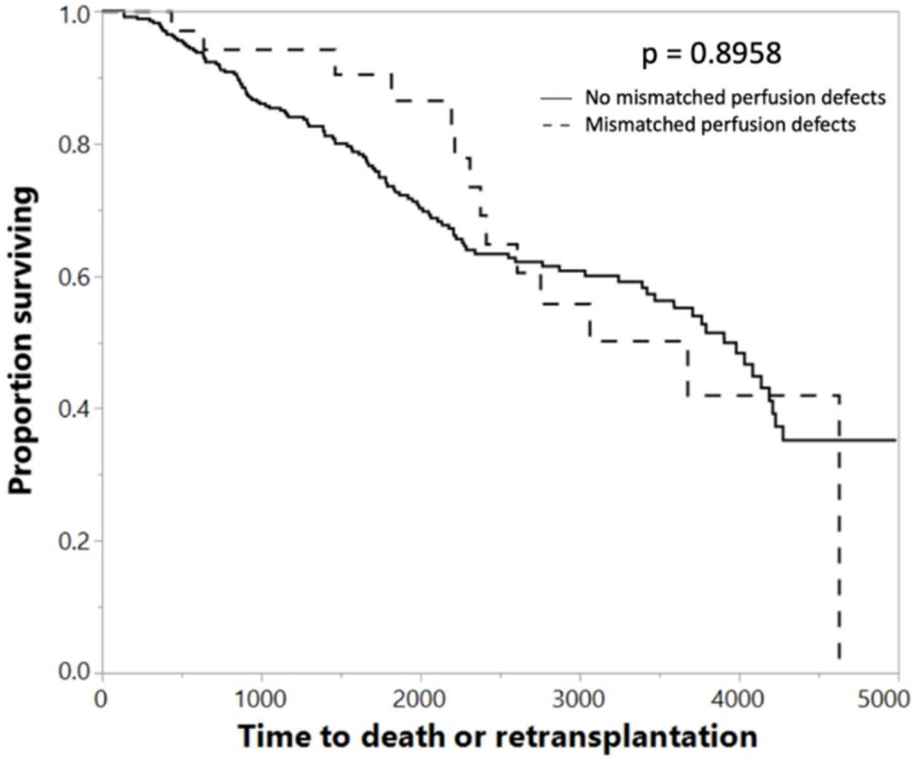

Figure 3. Kaplan Meier estimate of time to death or retransplantation after transplant stratified by patients with mismatched perfusion defects (dashed line) versus those with no perfusion defects (solid line) on 3-month routine ventilation perfusion scan.

Baseline graft function

Peak FEV1% predicted did not differ between the PD and no PD group (94% vs. 92%; p=0.58). BLAD was present in 144 patients (39%) after transplant but was no more frequent or severe in those with PD at 3 months compared to those without (Table 3). Therapeutic anticoagulation for possible PE as the cause of the perfusion defect did not alter this relationship (Table 4).

Table 4. Lung function and survival in the perfusion defect group stratified by therapeutic anticoagulation

Characteristic	Overall (n=35)	Treatment (n=7)	No treatment (n=28)	p-value
Baseline lung allograft dysfunction	14 (40)	3 (43)	11 (39)	1.000
Chronic lung allograft dysfunction	11 (31)	2 (29)	9 (32)	1.000
Max FEV1 % predicted, mean	94 (20)	91 (12)	95 (22)	0.445
1-year survival	35 (100)	7 (100)	28 (100)	1.000

Means are presented with standard deviations, medians with interquartile range and counts with percentages. FEV1 = forced expiratory volume in 1 second

Chronic lung allograft dysfunction

106 patients (28%) developed CLAD grade 1 or higher over the study duration. PD were not associated with CLAD onset risk (Figure 5). Anticoagulation for presumed PE did not alter this relationship (Table 4).

DISCUSSION

Perfusion defects on routine VQ scan were relatively common in our post-lung transplant cohort, occurring in 9% of patients. These changes were not associated with a difference in survival or lung function, irrespective of therapeutic anticoagulation.

The nature of what these defects represent is a critical question. PE has been shown to be relatively common in multiorgan donors, occurring in up to 30% in one cohort, as well as in mechanically ventilated patients in intensive care units, so residual clot in the donor

lungs could persist even following the anterior and retrograde flush.[3,17] However, it is also feasible these emboli are recipient-derived: post-operative transplant recipients fulfill all criteria for Virchow's triad with venous stasis due to edema and immobility, endothelial injury from surgery specifically involving the pulmonary blood vessels and hypercoagulability due to the post-operative state.[18] The vascular anastomoses could potentially give rise to thrombus in the pulmonary trunk as opposed to the lower extremities, meaning the loss of a potentially helpful clue and making PE more challenging to diagnose.[19,20] Few if any centers perform routine contrast-enhanced computed tomography studies in the early days of the post-operative course, during which there are many other potential causes of hypoxia, dyspnea and hemodynamic changes and when renal function can be tenuous. Altogether these factors make it difficult to ascertain the timeframe over which these clots have developed and most importantly whether they originated in the donor or the recipient. A potential avenue of future study would be prospective VQ scanning in the early post-operative phase – for example, two weeks post-transplant – in addition to the 3-month timepoint as this could help delineate the timing of these changes.

The presented results however could suggest these are donor-derived thrombus which occurred during donor management. Were these clots recipient-derived, we would expect to see serious clinical sequelae from a decision not to treat, namely a proportion of patients developing serious or fatal blood clots. This is due to anticoagulation for pulmonary embolism treating the state of hypercoagulability and the risk of a subsequent potentially fatal clot rather than the acute clot itself.[21] In our series, no patients in the PD

group were subsequently diagnosed with or admitted to hospital with serious PE and there were no sudden cardiac deaths. This would suggest that in the when these changes are detected on routine imaging, it is safe to monitor these patients in the absence of other indications for treatment such as DVT.

Another potential explanation for the lack of typical PE-associated prognostic change irrespective of therapy is that these VQ findings may not represent thrombus. Given these lungs are rigorously evaluated in the lead-up to transplantation, it is unlikely these changes reflect intrinsic chronic pulmonary pathology, especially at the frequency noted in this study. Fat embolism is another potential explanation, but this tends to be associated with major trauma and the development of acute respiratory distress, the combination of which would usually preclude organ donation.[22,23] Finally, vascular changes related to the surgery itself could be a potential cause of these changes. Given the focality and distribution of the mismatched VQ defects in this study, it is unlikely these relate to the vascular anastomoses themselves, but distal changes arising from the anastomoses are certainly possible.[24,25]

The lack of association between therapeutic anticoagulation as a response to these changes and 1-year survival or overall survival would suggest that treatment is not necessary or beneficial, particularly given untreated symptomatic PE bears a mortality of up to 30%.[26] We did not specifically observe harm related to therapy in those patients whom we elected to treat – most notably, no major bleeding events occurred – but the treatment group is small and not suited to addressing this. As noted, the treated patients

had additional findings to guide therapy, such as lower extremity deep venous thrombosis or documented Factor V Leiden mutation. Given the size of the risk group (n=35) and treatment group (n=7), the study is not well suited to definitively evaluating the role of therapeutic anticoagulation for these VQ findings, but conservative management involving observation and potential follow-up study does appear to be a viable strategy.

Chronic thromboembolic pulmonary hypertension (CTEPH) is a potential complication of both treated and untreated PE.[27] We performed routine follow-up with clinical assessment in all patients rather than routine echocardiography, in keeping with current recommendations given an estimated risk of the development of chronic thromboembolic pulmonary hypertension of 5%.[1,28] We observed no cases among the PD group where CTEPH was suspected and noted no association with peak FEV1 or baseline dysfunction risk, though we did note that all patients (n=6) who underwent follow-up VQ studies had residual changes suggestive of chronic PE. These findings suggest that these defects do not confer the same prognosis as conventionally diagnosed PE where investigations were triggered by symptoms, but the residual radiographic changes warrant further investigation.

Our study has limitations. First, we do not know the incidence or implications of these defects in patients who did not undergo VQ scanning because they died prior to 3 months post-transplant. It is possible some of these patients had serious enough perfusion defects to contribute to their outcome, but without a diagnostic study or routine post-mortem examinations (which our center does not perform), any estimates would be

speculative. Second, we did not feel we could rely on stratifying patients based on symptoms, given symptoms remain relatively common in this timeframe even in patients undergoing normal recovery and given the many competing risks of lung transplantation – infection, rejection, mechanical abnormalities – that can produce similar symptoms.[29] Third, given the differential sensitivities of a contrast computed tomography of the chest compared to a VQ scan, our study cannot clarify the prognostic or treatment implications of pulmonary emboli noted via that modality.[30] Fourth, SPECT imaging was not used for this study. SPECT utilization is increasing globally and has reported important differences in interpretation with higher diagnostic accuracy compared with planar scintigraphy. In our program, SPECT has become routine for VQ scans since 2016. Finally, it is important to note that this study's conclusions do not strictly apply to outpatients with a high clinical suspicion of acute pulmonary embolism.

Mismatched perfusion defects on routine post-transplant VQ scan are common but do not appear to confer an increased risk of death or graft dysfunction. A conservative approach including exclusion of DVT, monitoring and close follow-up of these patients appears to be a viable strategy and would be worthy of evaluation prospectively.

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CHAPTER 4 – Azithromycin Prophylaxis after Lung Transplant is associated with Improved Lung Function and Survival

INTRODUCTION

Chronic lung allograft dysfunction (CLAD) is the most important barrier to long-term survival after lung transplantation, accounting for approximately 40% of deaths after the first year.[1] CLAD is a syndrome manifesting in the airways and parenchyma of transplanted lungs that leads to a largely irreversible deterioration in lung function as measured by forced expiratory volume in 1 second (FEV1).[2] Despite its prevalence and implications, medical therapy has been underwhelming in its effectiveness and definitive treatment for advanced CLAD remains limited to retransplantation in select candidates.[3] Current medical treatments include chronic azithromycin therapy, changing calcineurin inhibitor from cyclosporine to tacrolimus, total lymphoid irradiation and extracorporeal photopheresis. Of these strategies, azithromycin has shown the most promise in attenuating and in some cases reversing the decline in graft function.[4,5]

Azithromycin is a macrolide antibiotic known to have anti-inflammatory properties and has demonstrated utility in several airway diseases, including the principal CLAD subtype. bronchiolitis obliterans syndrome.[5–10] In addition to treatment of established CLAD, a clinical trial showed that azithromycin prophylaxis (AP) in lung transplant recipients administered post-transplant at discharge improved lung function and reduced the composite endpoint of CLAD-free survival over a study period of 2 years.[11] However, there is no data to support an effect on overall survival or data from larger cohorts. There

are also concerns about both short and long-term toxicity with azithromycin therapy, including hearing impairment, bacterial resistance and cardiac arrythmias, though often still with net benefit.[12–14]

Our program implemented AP in 2010 based on the results of the above trial and have now used it routinely for 10 years. Our objective was to evaluate the association between routine use of chronic azithromycin therapy implemented prior to CLAD onset (AP) and overall survival compared to historic controls not receiving AP in a large cohort of lung transplant recipients. We also aimed to evaluate the relationship to risk of CLAD onset and baseline lung allograft dysfunction (BLAD).[15] We hypothesized that patients receiving AP would show improved survival and reduced rates of CLAD and BLAD.

METHODS

Study Population

We studied adult patients who underwent double lung transplant in the University of Alberta lung transplant program between January 1, 2004 and December 31, 2016. We excluded single-, heart-lung and lobar lung transplant recipients to be able to study baseline lung allograft dysfunction, currently only defined in double lung transplants. Double lung transplants constitute an overwhelming majority of cases in our program reflecting center preference. We also excluded patients who died <30 days post-transplant as well as those without detailed medication histories available.

Post-transplant management protocols

Current standard induction therapy in our program uses an interleukin 2-receptor antagonist (IL2RA) for standard patients with anti-thymocyte globulin being reserved for high immunologic risk patients with donor specific antibody at time of transplant. Induction therapy evolved over the study timeframe such that prior to 2010, the majority of patients received ATG as standard induction. Calcineurin inhibitor therapy with tacrolimus has been standard maintenance immune suppression in our program since approximately 2007, but in 2004-2006 there was still a small minority of patients receiving cyclosporine as first-line agent. Mycophenolate mofetil has been our standard cell cycle inhibitor since prior to 2004, with azathioprine used only in a small proportion of cases where mycophenolate is not tolerated. Patients who have positive flow cytometric crossmatch at time of transplant are treated with five cycles of plasmapheresis starting immediately post-operatively. Cytomegalovirus (CMV) prophylaxis with valganciclovir is stratified by CMV matching status, with CMV donor seropositive recipient seronegative patients receiving the longest prophylactic regimens between six and twelve months. Fungal prophylaxis evolved over the study timeframe from standard fluconazole to standard voriconazole for three months duration. Patients are followed typically twice per week for the first 3 months, monthly for the reminder of the first year and then every 3 to 6 months afterwards lifelong. Spirometry is obtained at all visits and complete lung function testing at yearly intervals.

Prophylactic azithromycin therapy

We defined AP as azithromycin 250 mg every other day *initiated prior to CLAD onset.* This is typically very soon after transplant and used chronically afterwards. We allowed

for inclusion of patients started later into their post-transplant course given the change in program strategy, providing they were started prior to CLAD onset. Patients who were started on AZM after CLAD onset were included in the comparison group.

Outcome Definitions

Complete survival and post-operative data were obtained from our prospectively maintained program database. The primary endpoint was time to death or retransplantation. We defined CLAD status, grade and subtype according to the 2019 ISHLT consensus criteria including thorough reviews of patient charts to rule out non-CLAD confounders.[2] BLAD was defined in accordance with our previously published definition as a failure to achieve both FEV1 and FVC ≥80% predicted on 2 consecutive spirometry tests at least 3 weeks apart in a double lung transplant recipient.[15]

Statistical Analysis

Continuous variables were summarized as means with standard deviations or medians with interquartile ranges and compared using t-tests or Wilcoxon tests depending on normality. Categorical variables were summarized as frequencies and percentages and compared using Fisher's exact test for binary categories, Pearson's chi-square for 3 or more categories, and Cochran Armitage trend tests for ranked variables. We analyzed overall survival using proportional hazards regression with AP as the variable of interest, adjusting for covariates selected on the basis of biologic plausibility, known relationships to transplant era, or demonstrated differences in baseline characteristics. These included recipient age, recipient body mass index, recipient sex, underlying diagnosis, induction

immune suppression, maintenance calcineurin inhibitor, intraoperative support modality (cardiopulmonary bypass, extracorporeal membrane oxygenation, or none), use of ventilation or extracorporeal membrane oxygenation bridging, donor age, and transplant era (2010 and prior, post-2010). We ensured there was a sufficient number of events to accommodate the included number of modeled variables to avoid overfitting via the 1 in 10 rule. All variables passed the proportional hazards assumption as evaluated by Schoenfeld residuals, including AP. We ran secondary unadjusted proportional hazards and logistic models to evaluate the association between AP and time to CLAD onset and post-transplant BLAD, respectively, as well as additional adjusted models accounting for group differences and transplant era. A p-value of 0.05 was considered significant. Analyses were performed on JMP 12 software (SAS Institute, Inc, Cary, NC) and R version 3.6.1 within R Studio version 1.2.5001 (Boston, MA).

RESULTS

485 patients underwent double lung transplant in our program between 2004 and 2016. A total of 40 patients (8%) lacked sufficient data for evaluation, 11 of whom died within 30 days post-transplant and were not eligible for AP while the remaining 29 did not have sufficiently detailed long-term medication records for evaluation (Figure 1). Of the 445 patients who met inclusion criteria, 344 (77%) received AP prior to CLAD onset with a median time to azithromycin of 51 days [25th-75th quartile 20-211]. Donor and recipient baseline characteristics were similar overall, but patients receiving AP were more likely to have received induction with IL-2 receptor antagonists (57% vs. 35%; $p<0.001$) and to

have been transplanted after 2010 (Table 1). Initial immune suppressive treatment with tacrolimus was more common in the AP group.

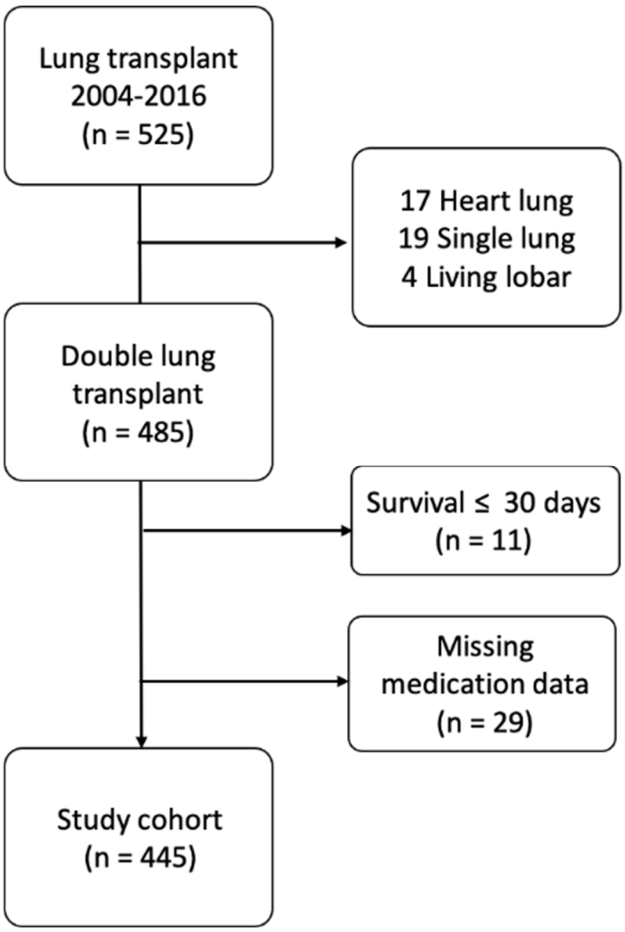


Figure 1. Study cohort.

Peri- and post-operative outcomes

Patients who received AP had shorter durations of mechanical ventilation (54 vs. 96 hours; p<0.001) but no difference in severe PGD. Total hospital stay was shorter in the AP group (23 [18-36] days vs. 29 [19-44] days; p=0.016).

Table 1. Baseline characteristics.

Characteristic	Overall (n=445)	Azithromycin prophylaxis (n=344)	No azithromycin prophylaxis (n=101)	p-value
Age in years, mean	52 ± 13	51 ± 14	53 ± 12	0.413
Female sex, n	154 (35)	120 (35)	34 (34)	0.905
BMI, mean	24.6 (5.0)	24.5 (5.0)	25.3 (5.0)	0.156
Diagnosis				
Bronchiectasis	69 (16)	58 (17)	11 (11)	0.196
Interstitial lung disease	166 (37)	120 (35)	46 (46)	
Obstructive lung disease	184 (41)	143 (42)	41 (41)	
Pulmonary vascular	17 (4)	15 (4)	2 (2)	
Other	9 (2)	8 (2)	1 (1)	
Recipient bridging				0.605
Ventilated	40 (9)	32 (9)	8 (8)	
ECMO	15 (3)	13 (4)	2 (2)	
None	390 (88)	299 (87)	91 (90)	
CMV mismatch	89 (20)	73 (21)	16 (16)	0.260
Cross match positive	57 (13) (n=425)	42 (13) (n=326)	15 (15) (n=99)	0.614
Intraoperative support				0.010
CPB	373 (84)	279 (81)	94 (93)	
ECMO	34 (8)	29 (8)	5 (5)	
None	38 (9)	36 (11)	2 (2)	
Induction therapy				**<0.001***

IL-2 receptor antagonists	232 (52)	197 (57)	35 (35)	
Anti-lymphocyte antibody	199 (45)	137 (40)	62 (61)	
None	14 (3)	10 (3)	4 (4)	
Maintenance therapy				
Tacrolimus	413 (93)	330 (96)	83 (82)	**<0.001***
MMF	441 (99)	340 (99)	101 (100)	0.579
Time to azithromycin in days, median	57 (20-283) (n=360)	51 (20-211)	1290 (1003-1804) (n=16)	**<0.001***
Transplant era pre-2010	210 (47)	134 (39)	76 (75)	**<0.001***
Donor				
Age in years, mean	39 (17)	39 (17)	37 (16)	0.223
Female sex	206 (46)	163 (47)	43 (43)	0.428
BMI, mean	25.4 (5.4)	25.5 (5.4)	24.9 (5.2)	0.281
Smoking > 20 pack years	60 (13)	47 (14)	13 (13)	1.000
Ischemic time in minutes, mean	352 (119)	347 (119)	365 (115)	0.188
Donor race				0.888
Caucasian	329 (74)	255 (74)	74 (73)	
Black	10 (2)	7 (2)	3 (3)	
Asian	22 (5)	16 (5)	6 (6)	
Other	84 (19)	66 (19)	18 (18)	

Means are presented with standard deviations, medians with interquartile range and counts with percentages. BMI = body mass index in kg/m2; CMV = cytomegalovirus; CPB = cardiopulmonary bypass; ECMO = extracorporeal membrane oxygenation; IL-2 = interleukin 2; MMF = mycophenolate mofetil

Table 2. Post-operative and long-term outcomes.

Characteristic	Overall (n=445)	Azithromycin prophylaxis (n=344)	No azithromycin prophylaxis (n=101)	p-value
Intubation time in hours, median	63 (33-171)	54 (29-150)	96 (48-245)	**<0.001***
Hospital LOS in days, median	23 (18-38)	23 (18-36)	29 (19-44)	**0.016***
Grade 3 PGD at 48 or 72 hours	76 (17)	63 (18)	23 (23)	0.312
BLAD	179 (40)	127 (37)	52 (51)	**0.011***
CLAD	129 (29)	91 (26)	38 (38)	**0.038***
Time to CLAD in days, mean	1397 (959)	1485 (1015)	1187 (782)	0.108
Max FEV1 % predicted, mean	92 (21)	93 (20)	87 (23)	**0.006***
Follow-up duration, median	1843 (1171-3149)	1798 (1171-3051)	2186 (1171-3453)	0.363

Table 3. Causes of death

Cause of death, n (%)	Overall (n=172)	Azithromycin prophylaxis (n=108)	No azithromycin prophylaxis (n=64)	p-value
Cause of death				0.4294
Acute rejection	3 (2)	3 (3)	0 (0)	
Cardiovascular	**17 (10)**	**11 (10)**	**6 (9)**	
CLAD	44 (26)	27 (25)	17 (27)	
Gastrointestinal	6 (3)	1 (1)	5 (8)	
Infection	35 (20)	22 (20)	13 (20)	
Malignancy	38 (22)	26 (24)	12 (19)	
Organ failure	18 (10)	11 (10)	7 (11)	
Other	9 (5)	6 (6)	3 (5)	
Unknown	2 (1)	1 (1)	1 (2)	

Overall survival

There were 170 deaths (38%) and one retransplant (0.2%) over the study duration. Given this, we felt it was justified to refer to this as overall survival rather than time to death or retransplantation. AP was associated improved overall survival (HR 0.60 [95% confidence interval [CI] 0.41-0.81]; p=0.002) in our adjusted model. We elected not to adjust for initial immune suppressive treatment with tacrolimus, as cyclosporine use was almost entirely 2010 and prior therefore accounted for by adjusting for transplant era. The AP group had higher one-year survival (99% vs. 91%, p<0.001). Unadjusted time-independent survival analysis via Kaplan Meier is depicted in Figure 2 for visualization purposes.

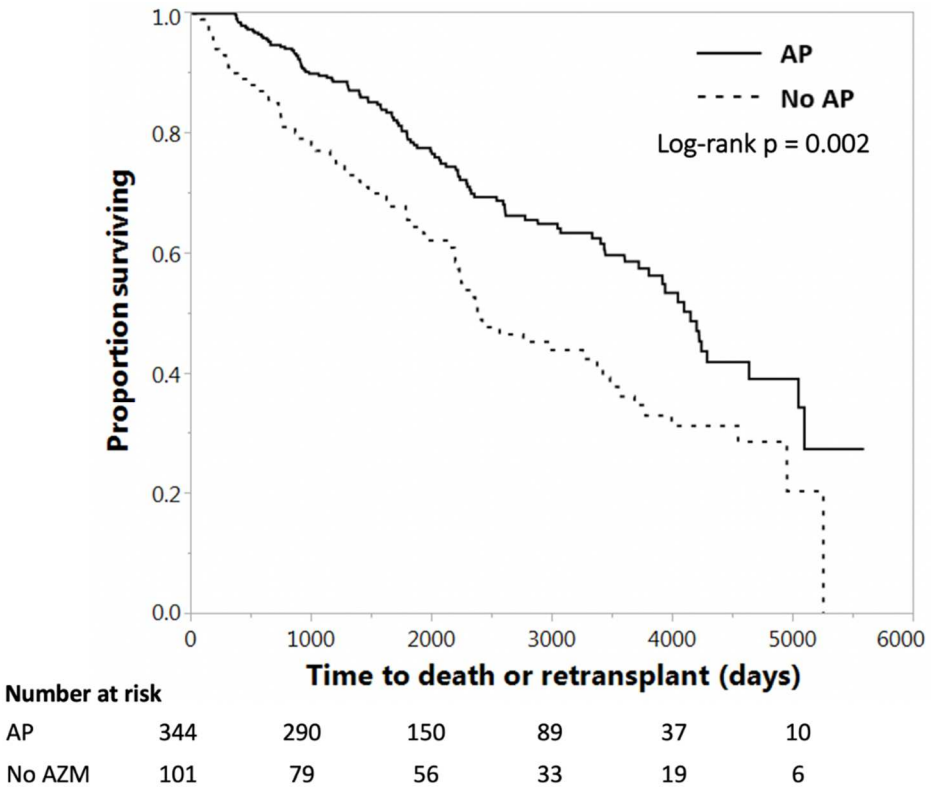
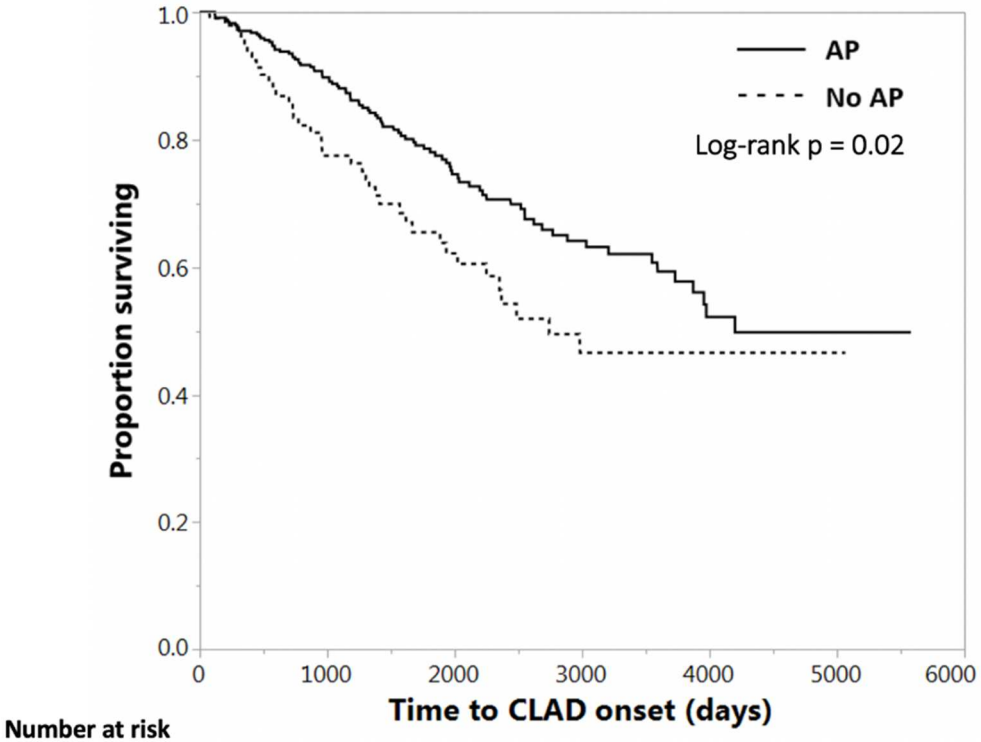


Figure 2. Kapan Meier estimate of time to death or retransplantation stratified by any prophylactic azithromycin use versus none. p-value reflects log rank test.

Azithromycin and the risk of CLAD and BLAD

AP was associated with a reduced unadjusted risk of CLAD onset (Figure 3; HR 0.64 [95% CI 0.44-0.94]; p=0.025), with AP treated as a standard binary variable as it did not violate the proportional hazards assumption in this model. AP was associated with a reduced unadjusted risk of post-transplant BLAD (OR 0.55 [95% CI 0.35-0.86]; p=0.009), as well as with less frequent severe grades of BLAD and CLAD (Figure 4).



Number at risk						
AP	344	270	123	68	28	9
No AZM	101	65	38	17	10	4

Figure 3. Kapan Meier estimate of death-censored time to CLAD1 onset stratified by any prophylactic azithromycin use versus none. p-value reflects log rank test.

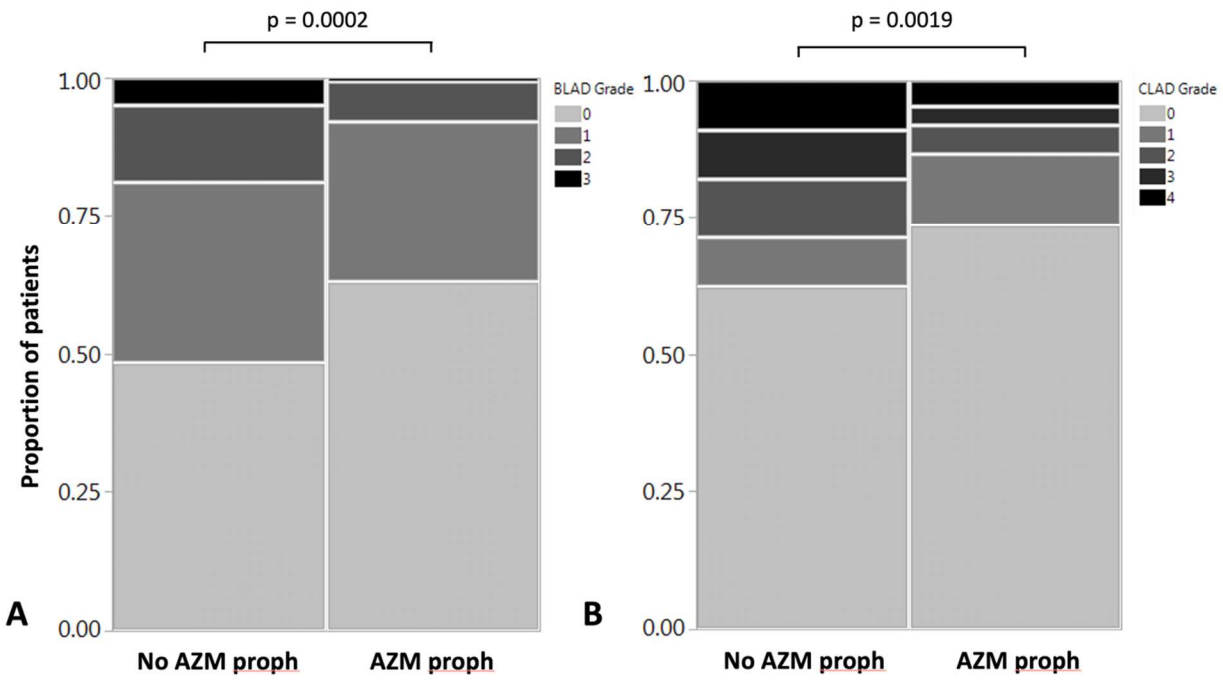


Figure 4. Distribution of BLAD (A) and CLAD (B) grades stratified by any prophylactic azithromycin use versus none. p-values reflect Cochran Armitage trend tests.

Azithromycin use and cardiovascular deaths

Cause of death data is illustrated in Table 3. Causes of death were not different in the AP group (p = 0.4294) versus the non-AP group. Specifically, we did not see an increase in the proportion of overall deaths attributable to cardiovascular events in this cohort (10% in AP vs. 9% non-AP).

DISCUSSION

Azithromycin prophylaxis was associated with improved overall survival in our large cohort of lung transplant recipients over an extended follow-up period. Secondary analyses were in keeping with prior trial results in showing a reduced CLAD risk in AP

recipients, but this is the first study first to our knowledge to describe an overall survival effect and a reduced risk of baseline dysfunction.

AP may confer survival benefits by multiple mechanisms. First, the development of CLAD is associated with increased mortality, so preventing or delaying CLAD onset may contribute.[16] CLAD is an inclusive term encompassing both bronchiolitis obliterans syndrome, restrictive allograft syndrome and a mixed disorder, but airways disease remains a core feature in most patients including those with RAS.[17,18] Azithromycin has immunomodulatory effects that have been postulated to play a role in its effectiveness in treating and preventing progression in inflammatory airway diseases.[7,8] In the initial trial from Vos et al., azithromycin reduced the composite endpoint of CLAD-free survival (that is, time to CLAD onset or death) but had no effect on overall survival.[4,19] The lack of an overall survival impact was attributed to the short follow-up time and the small sample size.[19,20] As such, our data can be seen as complementary to those findings. Of note, we found that AP was associated with a reduced unadjusted risk of CLAD onset but not after adjustment for group differences and transplant era ($p = 0.0870$), although we did note less severe CLAD grades in the AP group. These results suggest a role of AP in modifying CLAD severity, but that the survival benefits of AP may also be functioning through CLAD-independent mechanisms, namely reducing the risk of BLAD.

We have previously demonstrated that baseline lung allograft dysfunction – that is, failure to normalize lung function after transplant referent to population predicted values – is associated with increased risk of death independent of CLAD status.[15] Our results

indicate that AP was associated with reduced incidence and severity of BLAD, even after adjustment for era and induction immune suppression. We speculate that this reduced risk of BLAD in the AP group is contributing to the demonstrated survival benefit. This would be consistent with the cause of death data, where CLAD-attributed deaths were not more common in the no AP group. If AP was modifying the risk of BLAD, this would not necessarily reduce the risk of CLAD-related deaths specifically but, by augmenting baseline lung function, would attenuate the overall physiologic vulnerability of the cohort to all causes of death.

The presented study does not clarify the mechanisms by which azithromycin may mediate improvements in baseline post-transplant lung function, but we can speculate about several. A number of post-transplant injuries to the allograft could affect the ability of lung function to normalize, but inflammation related to resolving primary graft dysfunction, heavy donor smoking or early infection would be chief among them.[21] Early azithromycin has been shown to attenuate inflammation in a trial by Herck et al. showing a decrease in airway inflammation via lowered levels of inflammatory cytokines and neutrophilia. Neutrophils specifically are the primary innate cell involved in the PGD inflammatory cascade.[22, 23] Herck et al. did not demonstrate an improved FEV1 in patients receiving azithromycin at 3 months, but this measurement is distinct from our focus on baseline function, an outcome which is more inclusive by allowing lung function to normalize at any point after transplantation (median: 239 days in the overall cohort with no difference by AP treatment).

Treatment toxicity is an important consideration for new therapies and in particular in lung transplant recipients where it is already substantial.[24] This concern also exists with azithromycin, which has been associated with several toxicities including hearing loss and bacterial resistance in chronic use and ventricular arrythmia and cardiovascular death in pneumonia patients.[12,13] We did not observe a difference in the pattern of attributed causes of death in our cohort (Table 3) suggesting that this effect may not be a significant concern in a chronic stable setting. The relationship between short-term azithromycin therapy and ventricular arrythmia has also been questioned and suggested to be due to confounding, particularly when compared to other active antibiotics.[12,25] In addition to the cardiac effects, azithromycin has also been shown in some trials to result in sensorineural hearing loss, though rarely resulting in disability.[10] When used chronically in other respiratory disease such as cystic fibrosis however, this difference was not observed.[9] Our study lacks systematic data on auditory function, but in our experience we have not required frequent investigation or drug cessation for this indication.

Our study has limitations. First, the focus on a single center may incorporate center-specific effects that can limit generalizability. However, the effect remained consistent despite extensive adjustment for other factors, and our observed effects are consistent with prior studies and with biologic plausibility. Second, we chose to define AP as treatment initiated before CLAD onset. This was done to be as inclusive as possible and to differentiate from patients that were started on azithromycin for new CLAD onset. However, one could anticipate instances where the date of AP initiation and CLAD onset are temporally close, particularly where treatment is initiated because of early features of

CLAD without meeting the 2019 definition. However, of 91 AP patients who developed CLAD, the median time from AP initiation to CLAD onset was 988 days (IQR: 572–1,514 days) with only 5 patients having an AP to CLAD onset time of <90 days. This makes it unlikely that this definition is systematically misclassifying patients. As well, it should be noted that we allowed patients who were started on chronic azithromycin after CLAD onset into the control group (n = 16). Generally, allowing treatment effects in the comparison group tends to diminish the demonstrated effect estimate, so the persistence of a significant effect is further reassurance that it approximates the truth.[26] Fourth, our study lacks systematically collected data on auditory function or bacterial resistance patterns, both of which have been associated with chronic azithromycin therapy.[10] As above, our experience suggests this is an uncommon clinical concern but it remains a question worthy of further study. Finally, because this study utilizes a retrospective cohort, despite efforts to adjust, there remains a risk of residual and unmeasured confounding. Surgical and medical transplant practices have evolved over the study timeframe, both at our center and everywhere, which have resulted in improved post-transplant survival over time.[1,27–30] However, as noted, we have extensively adjusted both for variables that could have or were demonstrated to change over time as well as for the transplant era itself. We feel this is a meaningful methodology to account for era-dependent dynamics.

Our study demonstrated an overall survival benefit associated with azithromycin prophylaxis after lung transplant. This finding, in conjunction with the demonstrated effects on post-transplant lung function, pre-clinical studies on lung inflammatory cells,

and previous trial data, suggests that the benefits of AP warrant consideration for other lung transplant programs.

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CHAPTER 5 – Discussion

Identifying early markers for and ways to prevent CLAD – and all forms of post-transplant graft dysfunction – are priority areas for research in the current landscape of lung transplantation. The results of the three studies presented through this thesis allow us to draw some helpful conclusions that can help move forward our understanding of this field.

Our first objective was to assess relative lung perfusion in double lung transplant recipients at our center on routine lung VQ scans and their association with CLAD and mortality risk. Relative lung perfusion differential on VQ scans assesses the symmetry of pulmonary blood flow between the right and left lung, and also indirectly assesses ventilation due to the hypoxic pulmonary vasoconstriction response.[47] Interestingly however, marked relative lung perfusion differentials existed even in the absence of focal ventilation or perfusion defects on the scans. These patients with markedly unbalanced perfusion had impaired survival and increased risk for CLAD onset yet wide perfusion differential did not appear to be a simple reflection of poor spirometry either.

We speculate that wide perfusion differential may be a direct reflection of intrinsic abnormalities of the pulmonary vasculature which is differentially affecting the two lungs. In essence, perfusion differential could be capturing small, scattered matched perfusion defects unevenly distributed across the right and left lungs which are not otherwise detectable as focal lesions on imaging. In this way, perfusion differential would serve as a marker of heterogeneity which may present prior to frank radiographic findings or spirometry deteriorations in the development of CLAD. We rely on measuring airway

function via spirometry in the diagnosis of CLAD because it is what we can reliably test, but this does not mean CLAD as a disease process is confined to the airways.[4] Chronic rejection which is felt to be the main driver of CLAD takes place in the whole of the lung allograft and significant vascular manifestations of rejection have been previously demonstrated.[48,49] Moreover, the common loss of bronchial artery circulation with transplantation could make the allograft particularly vulnerable to insults to the remaining vasculature.[50] Prior studies leveraging MRI and specialized CT-based techniques have shown that perfusion abnormalities emerged prior to deterioration in spirometry in lung transplant recipients who went on to develop CLAD[21,22,51], and perfusion differential may be reflecting similar information through a less precise but more accessible imaging technique. This metric may serve as a novel early marker of risk for poor post-transplant outcomes and outlines a role for further investigation into the underlying mechanisms, particularly with respect to the involvement of the allograft vasculature in CLAD development. This could be done with protocolized repeat assessments of perfusion status via imaging techniques which we unfortunately did not have available for this study.

Another important feature of VQ scans is the presence of mismatched perfusion defects, which are classically associated with pulmonary vasculature occlusion and most typically pulmonary embolism. These were present in 9% of the cohort on routine 3-month VQ scans, and as such, it is important to evaluate their role as potential risk factors for future CLAD onset. This type of incidental finding presents a diagnostic and management challenge even in non-transplant populations for which the role for therapeutic anticoagulation remains unclear.[52] In lung transplant recipients, the implications of these

defects are further obscured by uncertainty as to whether they are donor or recipient derived emboli, or perhaps vascular changes related to surgery.[53] Donor-derived emboli would likely reflect low-risk lesions, as thrombus is remodeled and resorbed over time, providing it is not occlusive and life-threatening (which would have been the case in this cohort given these are routine 3-month scans as opposed to done for-cause). Recipient-derived emboli would be more problematic, as they would reflect a hypercoagulable state and a high risk for subsequent lung clots.[52] When weighed against the risks of therapeutic anticoagulation in this period however, the decision in our program has generally been conservative – monitoring as opposed to therapeutic anticoagulation – in the absence of frank clinical indications to treat. Aside from the risk for developing subsequent clots should these defects represent recipient derived emboli, chronic thromboembolic pulmonary hypertension (CTEPH) is a potential complication of both treated and untreated lung clots.[54] CTEPH is known to impair lung function and while perhaps not directly related to CLAD risk, it could certainly affect long term outcomes. Our findings would support this conservative approach: these mismatched perfusion defects incidentally found on VQ scans did not confer an increased risk of death or CLAD and BLAD irrespective of therapy. Further, the absence of serious clinical sequelae would suggest these defects are not true, de novo recipient-derived pulmonary emboli.

Our final objective was to test the utility of an azithromycin prophylaxis strategy for CLAD prevention[25] via a large retrospective cohort study. We sought to evaluate the impact of this prophylactic strategy on overall survival which had been identified as a limitation of the original study. The impact of an effective preventative therapy for CLAD cannot be

understated as there remains no effective, disease-modifying treatment for CLAD after onset.[4] Azithromycin administered as treatment again shows perhaps the most promise but there is no definitive evidence of sustained benefits when initiated in that context.[16] Though of note, there is a subset of patients with what has been termed “azithromycin responsive allograft dysfunction”, whose lung function recovers following treatment, but they do not fall under the CLAD umbrella by definition as their lung function decline is not persistent[4,26]. In brief, our study demonstrated an overall survival benefit associated with azithromycin prophylaxis after lung transplant. Importantly, we did not note any significant treatment toxicity associated with low dose chronic azithromycin. We additionally found that prophylactic azithromycin was associated with reduced risk for BLAD, suggesting that it could also be acting via augmenting baseline lung function, which would in turn attenuate the overall physiologic vulnerability of the cohort to all causes of death. While our study does not clarify the mechanisms of this association, we speculate that early administration of azithromycin could have the additional benefit of attenuating inflammation related to post-transplant allograft injuries which affect the ability of lung function to normalize. These injuries may include resolving PGD or early infection.[55] The exact mechanisms by which azithromycin acts to decrease lung inflammation remain under study but the association has been well-established in several pulmonary diseases, including COPD and cystic fibrosis.[56,57] Specifically in lung transplant recipients, early azithromycin has been shown to decrease airway inflammation via lowering levels of neutrophils and inflammatory cytokines.[58] This novel finding of improved post-transplant survival associated with azithromycin prophylaxis complements pre-clinical studies and

previous trial data in showing azithromycin as a safe and worthwhile preventative therapy for CLAD.

We have successfully identified a novel imaging feature which may be able inform clinicians and patients about the future risk of CLAD and may also provide insights into early changes in the at-risk transplanted lung. We also demonstrated for the first time that mismatched defects on routine studies can be safely conservatively monitored as opposed to treated. Finally, we showed that azithromycin prophylaxis was associated with improved overall survival, an effect likely at least in part mediated by improvements in lung function. These findings have implications for clinical practice. To build on the results of these studies, future directions include observational studies leveraging imaging modalities such as VQ scans, specialized CT-based techniques, or MRI for repeated longitudinal assessments of pulmonary vasculature function further into the post-transplant course. Given our speculations that azithromycin could be helping to attenuate early inflammation in the allograft, prospective studies assessing earlier initiation of azithromycin would be worthwhile.

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