Diffusing capacity of the lung for carbon monoxide: association with long-term outcomes after lung transplantation in a 20-year longitudinal study

David Ross Darley[1,2], Jin Ma[3], Ella Huszti[3], Rasheed Ghany[1], Michael Hutcheon[1], Chung-Wai Chow[1,4], Jussi Tikkanen[1], Shaf Keshavjee[1], Lianne Gail Singer[1,4] and Tereza Martinu[1,4]

[1]Toronto Lung Transplant Program, Toronto General Hospital, University Health Network, Toronto, ON, Canada. [2]UNSW Medicine, St Vincent's Clinical School, University of New South Wales, Sydney, Australia. [3]Biostatistics Research Unit, University Health Network, Toronto, ON, Canada. [4]Dept of Medicine, University of Toronto, Toronto, ON, Canada.

Corresponding author: Tereza Martinu (Tereza.Martinu@uhn.ca)

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In a cohort spanning 20 years, the D_{LCO} trajectory after lung transplantation is significantly associated with long-term outcomes including chronic lung allograft dysfunction and survival. https://bit.ly/3g3mvCk

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Abstract

Rationale The diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) measures gas movement across the alveolar–capillary interface. We hypothesised that D_{LCOcor} is a sensitive measure of injurious allograft processes disrupting this interface.

Objectives To determine the prognostic significance of the D_{LCOcor} trajectory on chronic lung allograft dysfunction (CLAD) and survival.

Methods A retrospective analysis was conducted of all bilateral lung transplant recipients at a single centre, between January 1998 and January 2018, with one or more D_{LCOcor} measurements. Low baseline D_{LCOcor} was defined as the failure to achieve a D_{LCOcor} >75% predicted. Drops in D_{LCOcor} were defined as >15% below recent baseline.

Results 1259 out of 1492 lung transplant recipients were included. The median (range) time to peak D_{LCOcor} was 354 (181–737) days and the mean±SD D_{LCOcor} was 80.2±21.2% pred. Multivariable analysis demonstrated that low baseline D_{LCOcor} was significantly associated with death (hazrd ratio (HR) 1.68, 95% CI 1.27–2.20; p<0.001). Low baseline D_{LCOcor} was not independently associated with CLAD after adjustment for low baseline forced expiratory volume in 1 s or forced vital capacity. Any D_{LCOcor} declines ⩾15% were significantly associated with death, independent of concurrent spirometric decline. Lower percentage predicted D_{LCOcor} values at CLAD onset were associated with shorter post-CLAD survival (HR 0.75 per 10%-unit change, p<0.01).

Conclusion Low baseline D_{LCOcor} and post-transplant declines in D_{LCOcor} were significantly associated with survival, independent of spirometric measurements. We propose that D_{LCOcor} testing may allow identification of a subphenotype of baseline and chronic allograft dysfunction not captured by spirometry. There may be benefit in routine monitoring of D_{LCOcor} after lung transplantation to identify patients at risk of poor outcomes.

Introduction

Graft survival after lung transplantation remains inferior to that demonstrated in other solid organ groups [1]. Longitudinal monitoring of allograft physiology after lung transplantation has been traditionally performed using the spirometric indices, forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC). Chronic lung allograft dysfunction (CLAD) is a major contributor to graft loss and is defined as the persistent and irreversible decline in FEV_1 [2]. Loss of lung volume, based on longitudinal volumetric monitoring, is the *sine qua non* for the restrictive allograft syndrome (RAS), a phenotype with important

clinical and prognostic implications [3, 4]. CLAD with gas trapping, based on an elevated residual volume to total lung capacity ratio, also predicts worse graft survival [5]. There is a paucity of research regarding the utility of alternative lung function tests in the definition of lung allograft dysfunction phenotypes and in prognostication.

The single-breath diffusing capacity of the lung for carbon monoxide (D_{LCO}) measures the capacity of the lungs to exchange gas across the alveolar–capillary interface. The D_{LCOcor}, corrected for haemoglobin ($mL \cdot min^{-1} \cdot mmHg^{-1}$), is the product of two simultaneous and separate measurements; the accessible alveolar volume and the rate constant for alveolar carbon monoxide uptake (K_{CO}) [6–10].

Current knowledge regarding the observed and predicted D_{LCO} measurements after lung transplant is based on early observational studies with limited sample sizes of between six and 34. Improvements in the post-operative percentage predicted D_{LCO} values were observed in single lung transplantation for COPD and idiopathic pulmonary fibrosis [11–13]. Larger improvements in D_{LCO} were observed after bilateral (in comparison with single) lung transplantation [14]. With regards to the D_{LCO} trajectory, maximal achieved values occurred at 12 months after transplant and appeared to decline over time [13, 15]. Reductions in D_{LCO} were observed during episodes of acute rejection and infection [16]. To the best of our knowledge, there have been no published studies to assess the effect of D_{LCO} measurements after lung transplantation on CLAD and survival.

We hypothesised that D_{LCO} is a sensitive measure of injurious allograft processes that can disrupt the alveolar–capillary interface and is a predictor of poor outcomes after lung transplantation. Our aims were to describe the trajectory of D_{LCO} measurements after lung transplantation, and to determine the prognostic significance of the D_{LCO} trajectory on CLAD and survival.

Methods

Subjects

A retrospective cohort analysis was conducted using a database of all lung transplant recipients at the Toronto General Hospital, encompassing 20 years between January 1998 and January 2018. Bilateral lung transplant recipients with one or more D_{LCO} measurement(s) were included. Recipients transplanted at other centres were excluded. Single lung transplant patients were excluded from all outcome analyses. The study was approved by the institutional research ethics board (protocol number 15-9531-AE).

D_{LCO} *measurements*

All study patients underwent measurement of D_{LCO} as a single-breath-hold 10-s manoeuvre, and reported as per American Thoracic Society guidelines [6, 17]. Daily checks for gas volumes, weekly syringe calibration and twice-monthly biological calibrations were performed for quality control.

Our hospital post-transplant lung function surveillance protocol includes D_{LCO}, with static lung volumes, at 3, 6, 9, 12, 18 and 24 months after transplantation and yearly thereafter. Values were obtained on the following commercial equipment at the Toronto General Hospital: Sensor Medics Vmax in 1997–1999, Morgan MDAS in 1999–2004 and Medisoft ExpAir 1.32.03 in 2004–2018. All observed D_{LCO} measurements were corrected for the nearest available haemoglobin value (D_{LCOcor}). The median (interquartile range (IQR)) time between D_{LCOcor} and closest haemoglobin measurement was 0 (2) days. The Global Lung Function Initiative reference values for D_{LCO} were used to generate predicted values [18].

Immunosuppression and CLAD treatment protocols

Refer to the supplementary material [19].

Clinical definitions

The baseline D_{LCOcor} was defined as the single maximum D_{LCOcor} value achieved after lung transplantation. Low baseline D_{LCOcor} was defined as the failure to achieve a baseline D_{LCOcor} >75% predicted. Drops in D_{LCOcor} were defined as declines ⩾15% below the best previously achieved value. Sustained declines were defined as irreversible reductions in D_{LCOcor}.

Clinical outcomes

The primary outcome was the time from transplant to the onset of CLAD (see supplementary material for definitions of CLAD and CLAD phenotypes) [2, 20]. Secondary outcomes included all-cause graft survival from transplant to death or re-transplantation and post-CLAD survival from CLAD onset to death or re-transplantation.

Statistical analysis

Descriptive statistics were summarised by mean±SD or median (IQR) for continuous variables, and counts (%) for categorical variables. To assess for significant differences between groups, the Chi-squared test was used for categorical variables and the Wilcoxon rank test for continuous variables. The D_{LCOcor} % pred trajectories were visualised with spaghetti plots to identify clinically relevant patterns. A multivariable logistic regression model was used to determine the association between baseline peri-operative variables and low first D_{LCOcor}, defined as below median % pred and measured ⩽4.5 months after transplant. Time-dependent multivariable Cox hazards models were used to determine the association between low baseline D_{LCOcor} and both CLAD and graft survival. For this analysis, the start point was the time of transplant, assuming that the patient was not in the low baseline D_{LCOcor} status until the first D_{LCO} measurement, and changing status with each subsequent measurement. Variables of interest potentially associated with CLAD and survival outcomes after transplantation were established *a priori* and included recipient age, donor age, donor–recipient sex matching, native lung disease, cytomegalovirus serostatus matching and transplantation era [1]. The landmark approach was adopted to adjust for spirometric decline (of ⩾12%) in the measurement of associations between low baseline D_{LCOcor} and both CLAD and graft survival [21]. Time-dependent multivariable Cox hazards models were used to determine the association between any in D_{LCOcor} and both CLAD and graft survival. Concurrent spirometric (FEV_1 or FVC) decline of ⩾12% at the time of each declined D_{LCOcor} was included as a covariate [22]. For declines analysis, all patients start at "no decline" status and remain so until the second D_{LCO} measurement, with their status changing based on the data points. A multivariable Cox proportional hazards model was used to determine the association between the D_{LCOcor} at CLAD onset and post-CLAD survival. Model specifications are summarised in the supplementary material. Statistical analyses were performed using R version 3.4.3 and Prism version 9.1.0. Statistical significance was set at a two-sided level of 0.05.

Results

D_{LCOcor} trajectory after transplantation

1723 patients underwent lung transplantation during the study period (figure 1). 231 patients were excluded from the study due to transplant not at Toronto General Hospital (n=6) or had fewer than one D_{LCOcor} measurement recorded (n=225). Of the remaining 1492 patients, 1259 were bilateral lung transplants and 233 were single lung transplants. The majority of patients (77.2%) completed the D_{LCOcor} surveillance as per protocol. The mean±SD baseline D_{LCOcor} value after single lung transplantation at 61.3±16.5% pred was lower compared to double lung transplantation at 80.2±21.2% pred. The differences are summarised in supplementary table S1 and supplementary figure S1. Single lung transplant recipients were excluded from all subsequent analyses, which focused only on the 1259 double lung transplant recipients with a total of 9543 D_{LCOcor} available measurements. The median (IQR) number of D_{LCOcor} measurements per patient was 7 (4–10). The median (IQR) days between D_{LCOcor} and closest haemoglobin measurement were 0 (0–2). Individual trajectories of D_{LCOcor} % pred values, visualised in figure 2, demonstrated high inter-patient variability. The median (range) time to peak D_{LCOcor} was 354 (181–737) days. For comparison, the median (IQR) time to peak FEV_1 was 278 (180–713) days.

Peri-operative determinants of the first D_{LCOcor} measurement

1074 patients had a first D_{LCOcor} measured ⩽4.5 months after transplant and the median first D_{LCOcor} was 68.4% pred. In a logistic regression analysis, increasing donor age by 5 years (OR 1.04, 95% CI 1.03–1.05; p<0.01), Canadian listing status 3 at transplant admission (OR 1.15, 95% CI 1.03–1.28; p=0.01), post-transplant intensive care unit (ICU) length of stay (OR 1.01, 95% CI 1.01–1.02; p<0.001) and primary graft dysfunction grades 2–3 (OR 1.11, 95% CI 1.01–1.21; p=0.02) were significantly associated with an increased risk of low first D_{LCOcor} (supplementary table S2). Higher A rejection scores, defined as the sum of all A grades divided by the number of available evaluable biopsies ⩽4.5 months after transplant, were protective for low first D_{LCOcor} (OR 0.92, 95% CI 0.86–0.97; p=0.004).

Baseline D_{LCOcor}

500 (39.7%) patients had low baseline D_{LCOcor} (<75% pred). There was a significant difference in the mean±SD baseline FEV_1 (66.8±19.0% pred) in patients with low baseline D_{LCOcor}, compared with 94.5 ±19.8% pred in those with normal baseline D_{LCOcor} (p<0.01). Recipient and donor characteristics measured at the time of transplant are summarised in table 1, comparing patients with low baseline D_{LCOcor} and those with normal baseline D_{LCOcor}.

Clinical outcomes

The median (IQR) overall follow-up time was 4.3 (2.0–8.2) years post-transplant. 588 (46.7%) out of 1259 patients met the criteria for a diagnosis of CLAD and 615 (48.8%) out of 1259 had no CLAD. In 56 (4.4%) out of 1259 recipients, there was lung allograft dysfunction without CLAD and the alternative

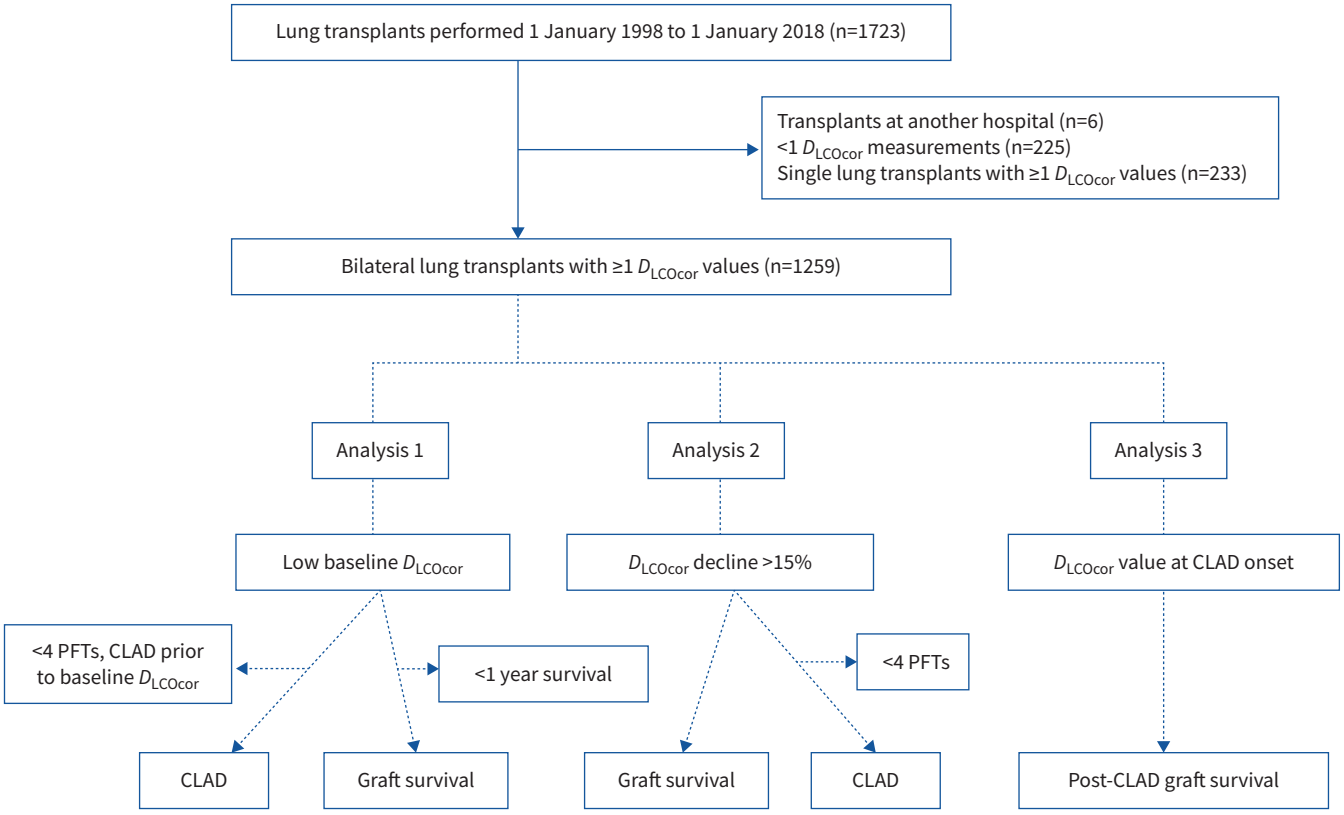

FIGURE 1 Flow diagram of analytic approaches in this observational study. D_{LCOcor}: diffusing capacity of the lung for carbon monoxide corrected for haemoglobin; CLAD: chronic lung allograft dysfunction; PFT: pulmonary function test.

diagnosis in all of these cases was infection. 546 (43.4%) out of 1259 died within the study period. Causes of death, available in 482 (88.3%) out of 546 patients, included CLAD (43.2%), bacterial sepsis (20.5%) and malignancy (12.0%).

Association between low baseline D_{LCOcor} and CLAD

In 116 recipients, a diagnosis of CLAD preceded the baseline D_{LCOcor} measurement and these were excluded from this analysis. Univariable analysis focusing on CLAD demonstrated that low baseline D_{LCOcor}, modelled as a time-dependent variable, was significantly associated with a shorter time to CLAD (hazard ratio (HR) 1.25, 95% CI 1.06–1.48; p=0.008). Multivariable analysis demonstrated that low baseline D_{LCOcor} was independently associated with a shorter time to CLAD (HR 1.29, 95% CI 1.08–1.55; p=0.005). The results are summarised in table 2. A landmark analysis was performed at 2 years post-transplant in order to adjust for baseline FEV_1 or FVC up to that pre-specified time point. Low baseline D_{LCOcor} at 2 years after transplant, adjusted for low baseline FEV_1 or FVC, demonstrated a positive correlation, albeit not statistically significant, with CLAD (supplementary table S3a). For visual illustration, figure 3a demonstrates a significant difference in the Kaplan–Meier curves for CLAD-free survival for patients with low baseline D_{LCOcor} compared to those with normal baseline D_{LCOcor}. To further illustrate the prognostic significance of D_{LCOcor} independent of spirometry, figure 3b shows the relative contribution of D_{LCOcor} to CLAD-free survival in the context of low or normal FEV_1. Even among patients with normal baseline FEV_1, those with low baseline D_{LCOcor} had reduced CLAD-free survival compared to those with normal baseline D_{LCOcor}. These findings were similar in the context of low or normal FVC (supplementary figure S3a).

Association between low baseline D_{LCOcor} and survival

In univariable analysis focusing on survival, low baseline D_{LCOcor}, modelled as time-dependent variable, was significantly associated with reduced survival (HR 3.26, 95% CI 2.64–4.01; p<0.001). In multivariable analysis, low baseline D_{LCOcor}, was independently associated with reduced survival (HR 3.42, 95% CI 2.76–4.25; p<0.001). The results are summarised in table 3. Landmark analysis of low

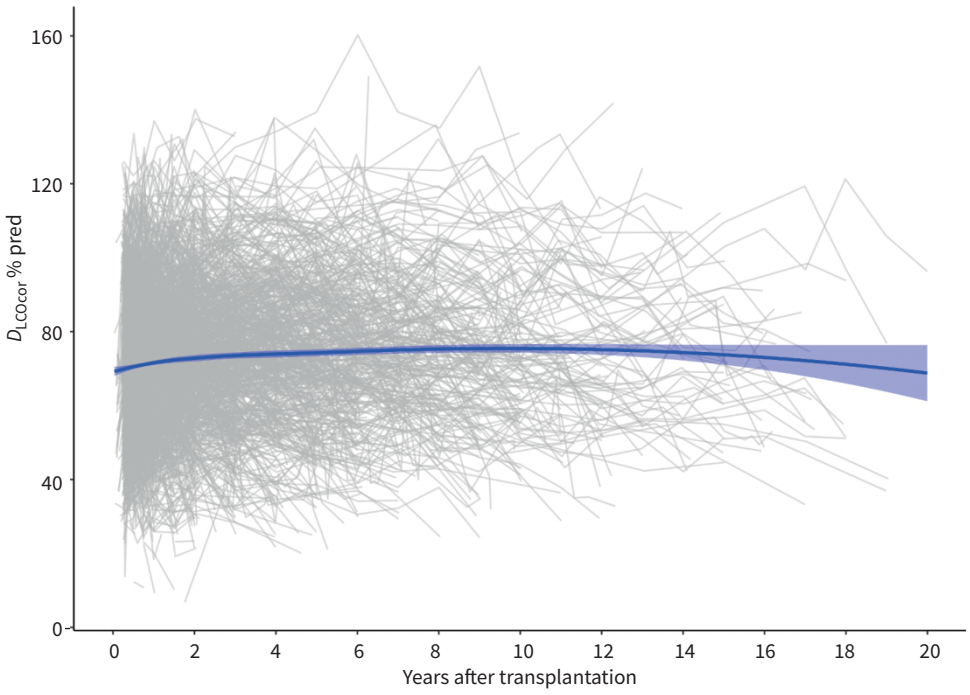

FIGURE 2 Spaghetti plot of the percentage predicted diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) trajectory after lung transplantation (n=1259) with high interpatient variability. The average trendline (95% confidence error) is highlighted in blue.

baseline D_{LCOcor} at 2 years after transplant showed that this association was independent of low baseline FEV_1 or FVC (supplementary table S3a). Figure 3c demonstrates a significant difference in the Kaplan–Meier curves for graft survival for patients with low baseline D_{LCOcor} compared to those with normal baseline $D_{LCOcor.}$ Figure 3d demonstrates the relative contribution of D_{LCOcor} on graft survival in the context of low or normal baseline FEV_1. Among patients with normal baseline FEV_1, patients with low baseline D_{LCOcor} had significantly worse survival than patients with normal baseline $D_{LCOcor.}$ Similar findings were observed in the context of low or normal baseline FVC (supplementary figure S3b).

Post-transplant declines in D_{LCOcor}

With regards to the D_{LCOcor} trajectory after the best achieved, 372 (31.0%) patients maintained stable values within 10% of best achieved. 829 (69.0%) patients demonstrated a sustained decline in D_{LCOcor} of ⩾10% from baseline. In 673 patients (56.0%), the sustained decline was ⩾15% below best achieved.

In 155 (12.9%) patients, a sustained decline in D_{LCOcor} occurred prior to CLAD onset with a median (IQR) lead time of 149 (73.8–307.5) days. In univariable analysis, any declines in D_{LCOcor} were significantly associated with CLAD (HR 1.39, 95% CI 1.14–1.69; p=0.001). In multivariable analysis after adjustment for concurrent FEV_1 or FVC decline, there was no significant association with CLAD (HR 1.06, 95% CI 0.84–1.34; p=0.63) (table 4). In univariable and multivariable analysis, any declines in D_{LCOcor} were independently associated with death (HR 2.49, 95% CI 1.97–3.15; p<0.001), even after adjustment for concurrent FEV_1 or FVC decline (table 4).

D_{LCOcor} at CLAD onset and post-CLAD survival

149 (12.4%) out of 1201 patients had a D_{LCOcor} measured within 30 days before or after CLAD onset. The median (IQR) D_{LCOcor} at at CLAD onset was 63.9 (50.4–77.2)% pred, significantly lower than the baseline value of 80.8 (69.8–90.4)% pred (p<0.01). In univariable analysis, the percent predicted D_{LCOcor} at CLAD onset was significantly associated with post-CLAD survival (HR 0.78 per 10%-unit increase, 95% CI 0.69–0.89; p<0.01). In multivariable analysis, adjusting for concurrent percentage predicted FEV_1 or FVC, lower D_{LCO} % pred values at CLAD onset remained independently associated with shorter post-CLAD

TABLE 1 Baseline recipient and donor demographics at the time of transplant, stratified by normal *versus* low baseline diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor})

	Normal baseline D_{LCOcor}	Low baseline D_{LCOcor}	p-value
Patients n	759	500	
Recipient age years	48.7±15.4	50.2±14.2	0.08
Donor age years	41.3±16.9	50.7±15.2	<0.001
Native lung disease			<0.001
COPD	229 (30.2)	102 (20.5)	
Cystic fibrosis	191 (25.2)	74 (14.9)	
Interstitial lung disease	185 (24.4)	224 (45.0)	
Other	154 (20.3)	98 (19.7)	
Sex-matching (recipient/donor)			0.07
F/F	246 (32.4)	171 (34.3)	
F/M	103 (13.6)	54 (10.8)	
M/F	98 (12.9)	86 (17.3)	
M/M	312 (41.1)	187 (37.6)	
CMV match status			0.28
D^-/R^-	201 (26.6)	112 (22.6)	
D^+/R^-	152 (20.1)	105 (21.2)	
R^+	404 (53.4)	279 (56.2)	
***Ex vivo* lung perfusion**	105 (13.8)	62 (12.4)	0.53
Transplant			0.22
First	738 (97.2)	477 (95.8)	
Second	21 (2.8)	21 (4.2)	
Transplantation era			<0.001
1998–2009	313 (41.2)	161 (32.2)	
2010–2018	446 (58.8)	339 (67.8)	

Data are presented as n, mean±SD or n (%), unless otherwise stated. F: female, M: male; CMV: cytomegalovirus; $D^{-/+}$: donor CMV seronegative/seropositive; $R^{-/+}$: recipient CMV seronegative/seropositive.

TABLE 2 Results of the time-dependent multivariable Cox hazards model assessing association of low baseline diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) with time to chronic lung allograft dysfunction (CLAD)

	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
Low baseline D_{LCOcor}	1.25 (1.06–1.48)	0.008	1.29 (1.08–1.55)	0.005
Native lung disease				
COPD	1.18 (0.91–1.54)	0.21	1.27 (0.97–1.67)	0.09
Cystic fibrosis	1.09 (0.83–1.44)	0.52	1.06 (0.80–1.40)	0.70
Interstitial lung disease	0.84 (0.64–1.10)	0.20	0.96 (0.74–1.26)	0.78
Other	Reference		Reference	
Sex matching (recipient/donor)				
F/M	1.07 (0.79–1.45)	0.65	1.16 (0.88–1.53)	0.28
M/F	0.97 (0.73–1.29)	0.86	0.98 (0.75–1.29)	0.90
M/M	1.20 (0.97–1.49)	0.09	1.24 (1.01–1.52)	0.04
F/F	Reference		Reference	
CMV serostatus (donor/recipient)				
D^+/R^-	1.52 (1.15–2.01)	0.003	1.61 (1.24–2.08)	<0.001
R^+	1.35 (1.08–1.70)	0.001	1.45 (1.16–1.80)	0.001
D^-/R^-	Reference		Reference	
Era of transplant				
2010–2018	0.95 (0.79–1.15)	0.62	0.93 (0.79–1.11)	0.44
1998–2009	Reference		Reference	
Recipient age (per 5-unit change)	0.99 (0.96–1.02)	0.53	0.96 (0.92–1.00)	0.03
Donor age (per 5-unit change)	1.03 (1.00–1.06)	0.04	1.03 (1.01–1.06)	0.01

Low baseline D_{LCOcor}, cytomegalovirus (CMV) serostatus, sex matching, recipient age and donor age were independently associated with a shorter time to CLAD. HR: hazard ratio; F: female, M: male; $D^{-/+}$: donor CMV seronegative/seropositive; $R^{-/+}$: recipient CMV seronegative/seropositive.

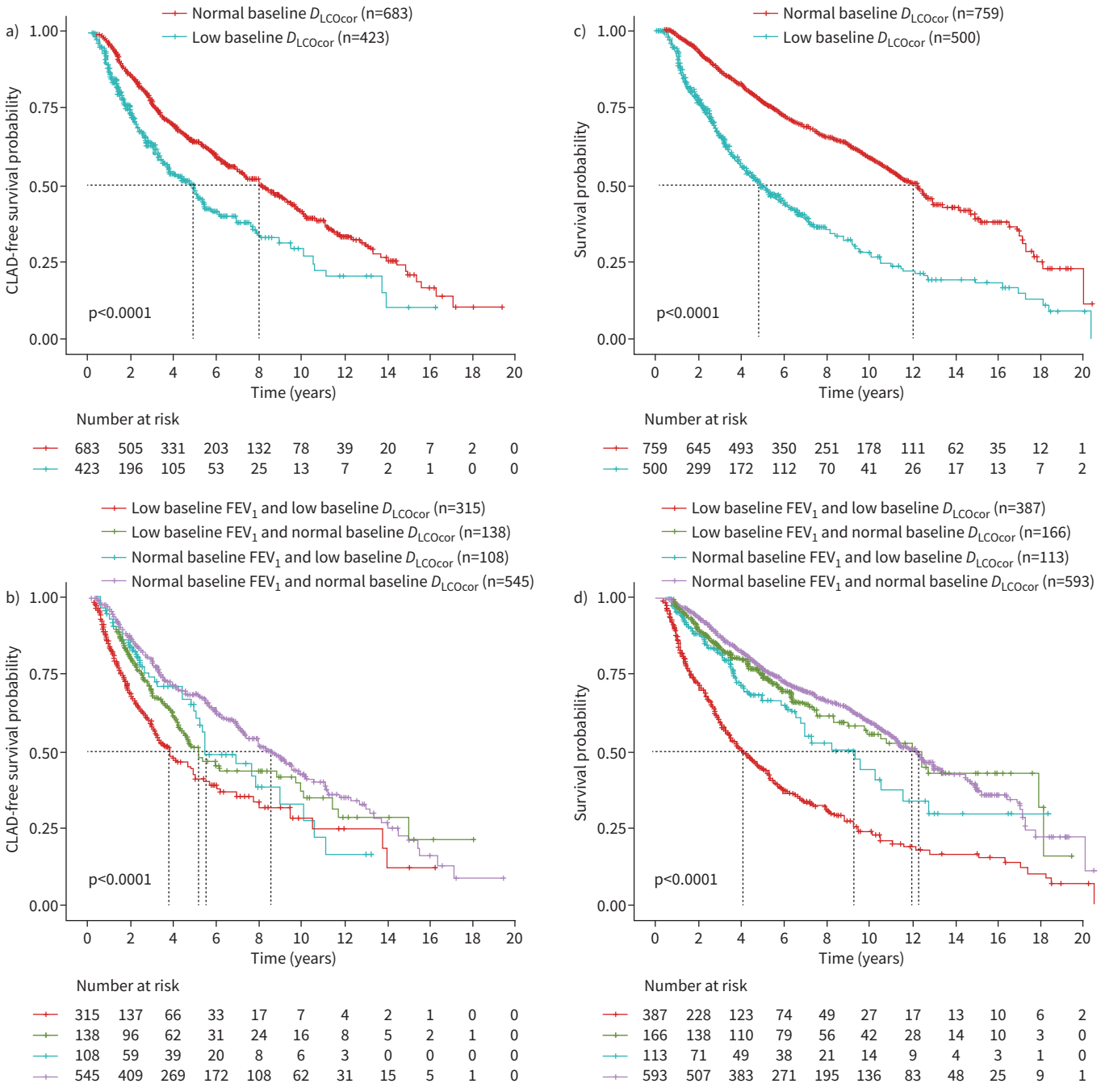

FIGURE 3 a) Kaplan–Meier curves for chronic lung allograft dysfunction (CLAD)-free survival. Patients with low baseline diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) (n=423) show significantly reduced CLAD-free survival compared to those with normal baseline D_{LCOcor} (n=683). b) Kaplan–Meier curves for four groups demonstrating the relative contribution of low or normal baseline forced expiratory volume in 1 s (FEV_1) and D_{LCOcor} on CLAD-free survival. Among patients with normal baseline FEV_1, those with low baseline D_{LCOcor} had reduced CLAD-free survival compared to those with normal baseline D_{LCOcor}. c) Kaplan–Meier curves for all-cause survival or retransplantation. Patients with low baseline D_{LCOcor} (n=500) demonstrate significantly reduced survival compared to those with normal baseline D_{LCOcor} (n=759). d) Kaplan–Meier curves for four groups demonstrating the relative contribution of low or normal baseline FEV_1 and D_{LCOcor} on graft survival. Among patients with normal baseline FEV_1, patients with low baseline D_{LCOcor} showed significantly worse survival than patients with normal baseline D_{LCOcor}. Dashed lines indicate median survival.

survival (HR 0.75 per 10%-unit change, 95% CI 0.66–0.86; p<0.01) (table 5). Figure 4a demonstrates a significant difference in the Kaplan–Meier post-CLAD survival curves, based on D_{LCOcor} % pred values at CLAD onset, dichotomised by a median cut-off.

D_{LCOcor} trajectories in CLAD phenotypes

Complete CLAD phenotyping with D_{LCOcor} data was available in 174 patients. This included bronchiolitis obliterans syndrome (BOS) in 104 (60%) recipients, RAS in 16 (9%), mixed CLAD in nine (5%), undefined in 14 (8%) and 31 remained unclassified (18%). There was a clinically significant difference in the post-transplant D_{LCOcor} % pred trajectory based on CLAD phenotype, with RAS, mixed and undefined showing greater decline than BOS and unclassified patterns (figure 4b). D_{LCOcor} measured at CLAD onset (±30 days) was available in 40 patients with complete CLAD phenotyping and was significantly lower in RAS and undefined compared to BOS ($p<0.01$) (supplementary figure S2) [20].

Discussion

Our results, from a large retrospective cohort of lung transplant recipients spanning a 20-year period, demonstrate a high inter-patient variability in the D_{LCOcor} trajectory after transplant. The post-transplant baseline diffusing capacity and any declines in D_{LCOcor} were associated with CLAD; however, this was not independent of the spirometric trajectory. The post-transplant baseline diffusing capacity, any declines in D_{LCOcor} and the percentage predicted D_{LCOcor} were independently associated with graft survival, importantly after adjustment for the spirometric trajectory.

Prior knowledge regarding the D_{LCOcor} after lung transplant was largely based on small, observational studies. Early studies demonstrated that a maximal achieved D_{LCO} occurred at 12 months after single lung transplantation [13, 15]. In our study we observed a median time to a best achieved D_{LCOcor} of 354 days after double lung transplant, longer than the median time to maximal FEV_1. To explain this period of physiological maturation, we hypothesise that prolonged recovery after implantation ischaemia–reperfusion, and/or early alloreactive injuries may also improve the K_{CO} over this time period [23]. Recovery from surgical trauma, thoracic pain and diaphragm weakness may improve the accessible alveolar volume during the first year.

Early studies demonstrated a significantly greater improvement in the observed D_{LCO} after double compared with single lung transplantation [14]. Our study confirms that higher baseline D_{LCOcor} % pred values are obtained after double lung transplant (81.3%) than those after single lung transplant (62.2%) [14]. This may be explained by the only partial correction in accessible alveolar volume after single lung transplant. Of interest, in our study only 16.1% of recipients achieved ⩾100% pred diffusing capacity after transplant. 39.7% of recipients never achieved a normal (>75%) baseline diffusing capacity. This finding is consistent

TABLE 3 Results of the time-dependent multivariable Cox hazards model assessing the association of low baseline diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) with time to death or retransplantation

	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
Low baseline D_{LCOcor}	3.26 (2.64–4.01)	<0.001	3.42 (2.76–4.25)	<0.001
Recipient age (5 units)	1.04 (1.01–1.07)	0.005	1.02 (0.97–1.06)	0.43
Donor age (5 units)	1.03 (1.01–1.06)	0.01	0.99 (0.96–1.02)	0.47
Native lung disease				
COPD	1.53 (1.19–1.97)	<0.001	1.54 (1.16–2.05)	0.003
Cystic fibrosis	1.09 (0.84–1.43)	0.52	1.30 (0.96–1.77)	0.09
Interstitial lung disease	1.40 (1.09–1.80)	0.008	1.23 (0.93–1.62)	0.15
Other	Reference		Reference	
Sex matching (recipient/donor)				
F/M	1.01 (0.77–1.34)	0.92	1.01 (0.76–1.35)	0.96
M/F	1.05 (0.81–1.37)	0.70	1.02 (0.78–1.34)	0.88
M/M	1.17 (0.96–1.43)	0.12	1.26 (1.02–1.55)	0.03
F/F	Reference		Reference	
CMV serostatus (donor/recipient)				
D^+/R^-	1.91 (1.47–2.47)	<0.001	1.77 (1.36–2.31)	<0.001
R^+	1.40 (1.12–1.76)	0.003	1.28 (1.01–1.62)	0.04
D^-/R^-	Reference		Reference	
Era of transplant				
2010–2018	0.87 (0.73–1.04)	0.13	0.88 (0.74–1.06)	0.18
1998–2009	Reference			

Low baseline D_{LCOcor}, native lung disease, sex matching and cytomegalovirus (CMV) serostatus were independently associated with a shorter time to graft loss. HR: hazard ratio; F: female, M: male; CMV: cytomegalovirus; $D^{-/+}$: donor CMV seronegative/seropositive; $R^{-/+}$: recipient CMV seronegative/seropositive.

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TABLE 4 Results of the time-dependent multivariable Cox hazards model for any declines in diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) and chronic lung allograft dysfunction (CLAD) or all-cause mortality including retransplantation

	Association with CLAD				Association with death			
	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
D_{LCOcor} decline	1.39 (1.14–1.69)	0.001	1.06 (0.84–1.34)[#]	0.63	3.64 (3.01–4.40)	<0.001	2.49 (1.97–3.15)[¶]	<0.001
FEV_1 decline	3.06 (2.49–3.76)	<0.001	3.00 (2.42–3.72)	<0.001	3.82 (3.05–4.78)	<0.001	2.67 (2.09–3.41)	<0.001
Recipient age (5 units)	0.99 (0.96–1.02)	0.53	0.92 (0.88–0.97)	<0.001	1.04 (1.01–1.07)	0.005	0.98 (0.93–1.03)	0.47
Donor age (5 units)	1.03 (1.00–1.06)	0.04	1.04 (1.01–1.06)	0.02	1.03 (1.01–1.06)	0.01	1.02 (0.99–1.05)	0.24
Native lung disease								
COPD	1.18 (0.91–1.54)	0.21	1.20 (0.88–1.64)	0.26	1.53 (1.19–1.97)	<0.001	1.03 (0.73–1.45)	0.87
Cystic fibrosis	1.09 (0.83–1.44)	0.52	1.02 (0.74–1.41)	0.89	1.09 (0.84–1.43)	0.52	0.96 (0.66–1.39)	0.83
Interstitial lung disease	0.84 (0.64–1.10)	0.20	1.10 (0.82–1.49)	0.53	1.40 (1.09–1.80)	0.008	1.15 (0.83–1.60)	0.40
Other	Reference		Reference		Reference		Reference	
Sex matching (recipient/donor)								
F/M	1.07 (0.79–1.45)	0.65	1.32 (0.96–1.81)	0.09	1.01 (0.77–1.34)	0.92	1.03 (0.72–1.47)	0.88
M/F	0.97 (0.73–1.29)	0.86	1.01 (0.74–1.38)	0.96	1.05 (0.81–1.37)	0.70	1.05 (0.75–1.46)	0.79
M/M	1.20 (0.97–1.49)	0.09	1.31 (1.04–1.65)	0.02	1.17 (0.96–1.43)	0.12	1.16 (0.90–1.49)	0.25
F/F	Reference		Reference		Reference		Reference	
CMV serostatus (donor/recipient)								
D^+/R^-	1.52 (1.15–2.01)	0.003	1.82 (1.36–2.45)	<0.001	1.91 (1.47–2.47)	<0.001	1.97 (1.43–2.70)	
R^+	1.35 (1.08–1.70)	0.001	1.49 (1.16–1.91)	0.002	1.40 (1.12–1.76)	0.003	1.33 (1.00–1.77)	<0.001
D^-/R^-	Reference		Reference		Reference		Reference	0.05
Era of transplant								
2010–2018	0.95 (0.79–1.15)	0.62	0.91 (0.75–1.11)	0.35	0.87 (0.73–1.04)	0.13	1.00 (0.79–1.25)	0.98
1998–2009	Reference		Reference		Reference		Reference	

Declines in D_{LCOcor} were adjusted for concurrent forced expiratory volume in 1 s (FEV_1) decline. HR: hazard ratio; F: female, M: male; $D^{-/+}$: donor CMV seronegative/seropositive; $R^{-/+}$: recipient CMV seronegative/seropositive. [#]: in multivariable analysis when D_{LCOcor} decline was adjusted for concurrent forced vital capacity (FVC) decline, there was no significant independent association with CLAD (HR 1.22, 95% CI 0.97–1.55; p=0.10); [¶]: in multivariable analysis when D_{LCOcor} decline was adjusted for concurrent FVC decline, there was a significant independent association with death (HR 2.34, 95% CI 1.84–2.98; p<0.001).

TABLE 5 Results of the final multivariable Cox proportional hazards model for diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) at chronic lung allograft dysfunction (CLAD) onset and post-CLAD survival (n=149)

	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
D_{LCOcor} % pred (10-unit increase)	0.78 (0.63–0.87)	<0.01	0.75 (0.66–0.86)	<0.01
FEV_1 % pred	0.99 (0.97–1.00)	0.16	0.99 (0.97–1.01)	0.15
FVC % pred	0.98 (0.96–1.00)	0.02	1.00 (0.98–1.03)	0.99

D_{LCOcor} % pred values at CLAD onset were independently associated with time to post-CLAD graft loss. HR: hazard ratio; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity.

with studies examining the spirometric trajectory after lung transplant [24]. There are probable alloimmune and non-alloimmune injurious processes which may never allow the allograft to recover fully after lung transplant.

We examined perioperative variables associated with low first D_{LCOcor}. The risk associated with increasing donor age may be explained by previous work demonstrating age-related declines in diffusion capacity independent of alveolar volume, suggesting alterations of the alveolar–capillary membrane [25]. Urgency listing status at transplant admission and post-transplant ICU length of stay are both markers of physiological vulnerability to allograft injury in the peri-operative period. High-grade primary graft dysfunction and need for post-transplant extra-corporeal membrane oxygenation probably reflect ischaemia–reperfusion injury of the transplanted lungs. Our findings are in keeping with previous work examining functional outcomes using cardiopulmonary exercise testing after lung transplant. In that study, lung volumes and diffusion capacity were significantly lower for recipients with grade 3 primary graft dysfunction within 72 h compared to those without [26]. We hypothesise that ischaemia–reperfusion may cause persistent disruption of the alveolar–capillary interface affecting gas transfer, and ventilatory restriction affecting the accessible alveolar volume. Unexpectedly higher A rejection scores were protective for low first D_{LCOcor}. We hypothesised that recipients undergoing early transbronchial biopsy surveillance may select for a cohort with higher earlier D_{LCOcor} values. While the association remained significant, there was a significant change in the odds ratio for low D_{LCOcor} after adjustment for the number of biopsies the recipient underwent in the first 4.5 months. We believe that this result is hypothesis-generating. The association between D_{LCOcor} and acute rejection deserves further attention with an appropriately designed study.

A key finding in our study is that recipients with low baseline D_{LCOcor} are at increased risk of CLAD and early graft loss. This indicates a "horse-racing" effect, the concept that low baseline values predict future low lung function and reduced graft longevity [27, 28]. Increased physiological vulnerability to cumulative post-transplant injurious processes may explain these associations. Baseline lung allograft dysfunction, defined as the failure to achieve both FEV_1 and FVC ⩾80% pred after transplant, has previously been shown to be a dynamic risk state associated with reduced graft survival [24]. Importantly, the D_{LCOcor} effect on survival that we see in our study is independent of FEV_1 and FVC. We demonstrate that recipients with low baseline D_{LCOcor} and normal baseline FEV_1 showed significantly worse overall graft survival than patients with normal baseline D_{LCOcor} and normal baseline FEV_1 and also worse compared to patients with normal baseline D_{LCOcor} and low baseline FEV_1. Results were similar when analysing FVC in a similar fashion. This underscores the potential importance of incorporating the baseline percentage predicted D_{LCOcor} value, as an additional metric to FEV_1 and FVC, for physiological phenotyping and prognostication after lung transplant.

The majority of recipients (69.0%) demonstrated a sustained decline of ⩾15% in D_{LCOcor} in relation to baseline values at some point during their post-transplant trajectory. The absence of an independent association between any D_{LCOcor} decline and subsequent CLAD is of interest. A wide range of pathological processes may cause a reduction in either the accessible alveolar volume or gas transfer. Some of these phenomena may represent non-CLAD causes of allograft dysfunction, or intercurrent pathological processes in patients with CLAD, underscoring the importance of clinical interpretation. We argue that D_{LCOcor} decline and spirometric decline appear to be related metrics of CLAD. In a cross-sectional study, the diffusion capacity for nitrous oxide allowed for early detection of BOS, and this requires further longitudinal prospective evaluation [29]. Any D_{LCOcor} decline was independently associated with reduced survival. D_{LCOcor} decline likely represents increased physiological vulnerability to biological processes leading to reduced patient survival.

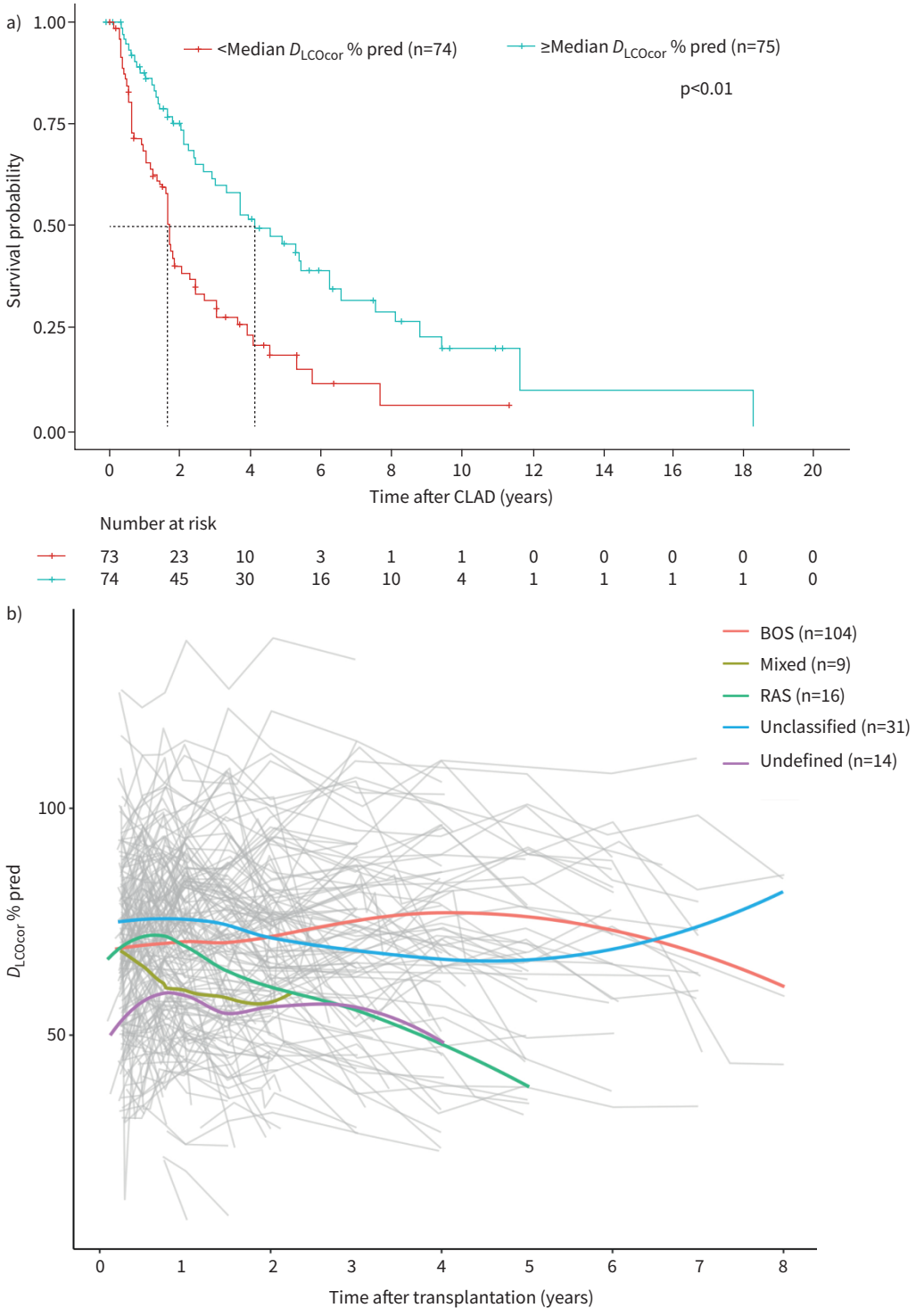

FIGURE 4 a) Diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (D_{LCOcor}) % predicted values at chronic lung allograft dysfunction (CLAD) onset were dichotomised with a median cut-off. Kaplan–Meier curves show significantly reduced post-CLAD survival for those patients with D_{LCOcor} % pred below median (n=74) *versus* those patients with D_{LCOcor} % pred above median (n=75). Dashed lines represent median survival. b) Spaghetti plot of the D_{LCOcor} % pred trajectories based on CLAD phenotype (n=174). The average trendlines are highlighted. There is a clinically significant difference in the post-transplant D_{LCOcor} % pred trajectory based on CLAD phenotype, with restrictive allograft syndrome (RAS), mixed and undefined showing greater decline than bronchiolitis obliterans syndrome (BOS) and unclassified patterns.

The percentage predicted D_{LCOcor} at CLAD-onset predicted post-CLAD survival, independently of concurrent spirometric decline. Significant injury of the alveolar–capillary interface, affecting the transfer factor, is the likely explanation for this finding, independent of the accessible alveolar volume. Thus, we argue that the diffusing capacity may provide additional information to assist clinicians in prognostication of recipients with a diagnosis of CLAD.

There was a clinically significant difference in the D_{LCOcor} trajectories based on established CLAD phenotypes with lower values in patients with restriction and fibrosis-like opacities on radiology. Further work is required to define the early inciting injurious allograft processes affecting the accessible alveolar volume and alveolar–capillary interface leading to CLAD and the fibrotic CLAD phenotypes. Whether D_{LCOcor} may be helpful in subphenoptying of CLAD should be explored in future studies with larger numbers of patients with relevant measurements.

Our study spans a long period of time and encompasses multiple transplant eras. During this time there have been significant alterations to transplant practice including immunosuppression, use of *ex vivo* lung perfusion and clinical phenotyping. We observed a greater proportion of patients with low baseline D_{LCOcor} in the latter era. However, transplant era was included as a covariate in all of our multivariable models and did not act as a significant confounder to the associations presented in this report.

There are limitations to the results presented in our study. Our study population is biased towards a healthier cohort, namely those patients who could perform one or more D_{LCOcor} measurements. Technical limitations with D_{LCOcor} measurements in recipients with advanced allograft dysfunction may have limited the number of measurements in the lowest range. We noted high inter- and intra-patient variability in diffusion capacity measurements. High inter-session variability in the measurement of D_{LCOcor} in healthy individuals, which is dependent upon the baseline D_{LCOcor} and method of testing used, has been demonstrated previously [30]. The use of three different commercial equipments over the 20-year period may also contribute to variability in observed values. Quality control was consistent over the 20-year period including daily checks for gas and volumes, twice monthly biological calibrations and monthly analyser linearity for primary standard gases. Given the consistency in quality control over the past 20 years, we believe that our results were consistent and comparable. Further work is required to elucidate the intersession variability in D_{LCOcor} after lung transplantation.

Conclusion

The post-transplant baseline diffusing capacity and any declines in D_{LCOcor} were associated with CLAD; however, this was not independent of the spirometric trajectory. The post-transplant baseline diffusing capacity, any declines in D_{LCOcor} and the percentage predicted D_{LCOcor} were independently associated with graft survival, importantly after adjustment for the spirometric trajectory. Routine monitoring of D_{LCOcor} after lung transplantation may improve the identification of patients at risk of poor outcomes.

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