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Alveolar-capillary membrane conductance and clinical outcomes in fibrotic interstitial lung disease

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Article

Keywords: Interstitial lung disease, diffusion capacity, nitric oxide, prognostic

Posted Date: August 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10621956/v1

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Additional Declarations: No competing interests reported.

Abstract

Background The clinical utility of single-breath diffusing capacity for nitric oxide (DLNO) and its derived components, alveolar-capillary membrane conductance (DmCO) and pulmonary capillary blood volume (Vc), remains incompletely defined in fibrotic interstitial lung diseases (ILDs). We therefore assessed the measurement properties of DLNO-derived indices and explored their associations with clinical outcomes in idiopathic pulmonary fibrosis (IPF) and systemic sclerosis-associated ILD (SSc-ILD).

Methods In this prospective multicentre cohort study, adults with IPF or SSc-ILD underwent simultaneous DLNO and DLCO measurements at baseline and 24 weeks. DmCO and Vc were derived using the Roughton–Forster model. A randomly selected subgroup underwent repeat testing after 24 days. Repeatability, construct validity, responsiveness, associations with clinical outcomes, and preliminary estimates of clinically meaningful change were evaluated.

Results Ninety-three patients were included (73 IPF and 20 SSc-ILD). DLNO was proportionally more impaired than DLCO, reflecting reductions in both DmCO and Vc. DLNO-derived measurements showed good repeatability (intraclass correlation coefficients > 0.80) and significant associations with physiological indicators of disease severity. Changes over 24 weeks correlated modestly with changes in dyspnoea. Among all evaluated parameters, DmCO decline showed the most consistent associations with transplant-free survival, mortality, and respiratory hospitalization. Estimated thresholds for clinically meaningful change (% predicted) were 3.1 for DLNO, 4.9 for DmCO, and 7.3 for Vc.

Conclusions: DLNO-derived measurements, particularly DmCO, demonstrated good reproducibility, physiological validity, and responsiveness to longitudinal change in fibrotic ILD. Associations between DmCO decline and clinical outcomes suggest potential clinical relevance and warrant confirmation in larger prospective studies.

Clinical trial number: not applicable.

INTRODUCTION

Gas exchange impairment is a hallmark of fibrotic interstitial lung diseases (ILDs) and a major determinant of symptoms, exercise limitation, and clinical outcomes [1]. Idiopathic pulmonary fibrosis (IPF) and systemic sclerosis-associated ILD (SSc-ILD) represent distinct fibrotic ILD phenotypes that share disruption of the alveolar-capillary unit and impaired pulmonary gas transfer. Progressive alterations of the alveolar-capillary membrane and pulmonary microvasculature contribute to worsening oxygen transport and functional decline [2, 3].

The diffusing capacity of the lung for carbon monoxide (DLCO) is widely used to assess gas-transfer abnormalities in ILD and is an established marker of disease severity and prognosis [4, 5]. However, DLCO provides a global estimate of pulmonary diffusing capacity and cannot distinguish between abnormalities arising from the alveolar-capillary membrane and those related to the pulmonary vascular compartment. Consequently, different pathophysiological mechanisms may result in similar DLCO values.

The single-breath diffusing capacity for nitric oxide (DLNO) has emerged as a complementary measure of pulmonary gas exchange [6–8]. Because nitric oxide binds haemoglobin substantially faster than carbon monoxide, DLNO is more closely related to alveolar-capillary membrane conductance (DmCO) [9, 10], while the combined assessment of DLNO and DLCO allows estimation of both DmCO and pulmonary capillary blood volume (Vc) [11]. This partitioning may provide a more detailed physiological characterization of gas-transfer impairment in fibrotic lung disease.

Despite its physiological appeal and increasing availability [9], the clinical role of DLNO-derived measurements in ILD remains incompletely defined. Previous studies have reported reduced DLNO values in fibrotic lung diseases and suggested potential clinical relevance [12–14], but most investigations have been limited by small sample sizes, cross-

sectional designs, or methodological heterogeneity. Moreover, key measurement properties, including repeatability, construct validity, responsiveness to change, and clinical interpretability, have not been comprehensively evaluated in prospective cohorts of patients with fibrotic ILD.

Therefore, we aimed to characterize the measurement properties of DLNO-derived indices, including DmCO and Vc, in patients with IPF and SSc-ILD. Specifically, we evaluated their repeatability, construct validity, and responsiveness to longitudinal change, explored their associations with clinically relevant outcomes, and estimated preliminary thresholds for clinically meaningful longitudinal change.

METHODS

Study design and participants

This prospective multicentre longitudinal cohort study was conducted at four tertiary referral centres in Spain. Eligible participants were adults aged 18–80 years with a confirmed diagnosis, established within the previous two years, of either idiopathic pulmonary fibrosis (IPF) or systemic sclerosis-associated interstitial lung disease (SSc-ILD). IPF was diagnosed according to contemporary ATS/ERS/JRS/ALAT recommendations [15], requiring a usual interstitial pneumonia pattern on high-resolution computed tomography (HRCT) or surgical lung biopsy and exclusion of alternative causes of fibrosis. SSc-ILD was defined in patients fulfilling American Rheumatism Association criteria for systemic sclerosis [16] and presenting HRCT evidence of interstitial lung involvement.

Detailed inclusion and exclusion criteria are provided in the online supplementary material. Patients were withdrawn from the study after lung transplantation or withdrawal of informed consent. The study was approved by the institutional ethics committee in accordance with the Declaration of Helsinki and all patients provided written informed consent.

The primary objective of the study was to evaluate the measurement properties of DLNO-derived indices, including repeatability, construct validity, and responsiveness to longitudinal change. Associations between changes in DLNO-derived measurements and subsequent clinical outcomes were evaluated as secondary exploratory analyses.

Measurements and follow-up

Participants attended study visits at baseline and after 24 weeks. In addition, a randomly selected subgroup of 40 participants underwent repeat testing after 24 days to assess short-term repeatability under clinically stable conditions. Random selection was performed using computer-generated allocation (R software version 4.1.2). Clinical management was not modified by study participation. Follow-up telephone contacts were performed at 12 and 24 months after the 24-week visit.

Clinical outcomes evaluated during follow-up included transplant-free survival, all-cause mortality, respiratory hospitalisations [17], and acute exacerbations. Transplant-free survival was defined as survival without lung transplantation from the week-24 assessment onwards.

At each visit, demographic and clinical data were recorded, including ILD subtype, smoking status (never, former, or current smoker), cumulative tobacco exposure, comorbidities, previous hospitalizations, and ongoing treatments, including oxygen therapy, corticosteroids, immunomodulatory agents, biologics, and antifibrotic drugs. Dyspnoea was assessed using the modified Medical Research Council (mMRC) scale and the Baseline/Transition Dyspnoea Indexes (BDI/TDI) [18]. Health-related quality of life was evaluated using the 36-item Short Form Health Survey (SF-36) [19].

Arterial blood gases were obtained under resting conditions while breathing room air whenever clinically feasible. Exercise capacity was assessed using the six-minute walk test (6MWT) performed according to American Thoracic

Society recommendations [20]. Baseline transthoracic echocardiography was performed following ASE/EACVI recommendations, including estimation of pulmonary artery systolic pressure and standard right-heart measurements [21].

Pulmonary function testing

All pulmonary function measurements were performed at a single reference laboratory to ensure methodological consistency. Spirometry and single-breath diffusing capacity for carbon monoxide (DLCO) were obtained using a MasterLab 6.0 system (Viasys, Würzburg, Germany) following international recommendations [22, 23]. Predicted values were calculated according to Global Lung Function Initiative reference equations [24, 25]. The Composite Physiologic Index (CPI) was calculated using the published formula [26].

After a 20-minute resting period, diffusing capacity for nitric oxide (DLNO) was measured using the single-breath technique according to European Respiratory Society technical standards [9]. The inspired gas mixture contained approximately 40–50 ppm nitric oxide, 0.3% carbon monoxide, 9% helium, 20.9% oxygen, and balance nitrogen. Participants inhaled rapidly from residual volume to total lung capacity, maintained a breath-hold of approximately five seconds, and subsequently exhaled in a controlled manner. Continuous measurements of nitric oxide, carbon monoxide, helium concentration, and exhaled volume were recorded. Quality-control criteria required an inspired volume of at least 90% of the largest measured vital capacity, absence of Valsalva or Müller manoeuvres, and at least two reproducible measurements within 10% or 2 $mL \cdot min^{-1} \cdot mmHg^{-1}$, whichever was greater. A maximum of five attempts was allowed, and the average of the two highest reproducible values was recorded.

Simultaneous determination of DLNO and DLCO enabled partitioning of pulmonary diffusing capacity into alveolar-capillary membrane conductance (DmCO) and pulmonary capillary blood volume (Vc) according to the Roughton–Forster model [11]. Results were expressed both as absolute values and as percentages of predicted values using published reference equations.

Additional technical details are provided in the online supplementary material.

Statistical analysis

Sample size estimation was based on published short-term DLCO decline in ILD [27], assuming DLNO would show approximately twice the magnitude of change. Under these assumptions, 93 participants provided 80% power ($\alpha = 0.05$) with 10% attrition. The study was not specifically powered for survival or other clinical outcome analyses, which should be considered exploratory.

Continuous variables are presented as mean ± standard deviation or median (first quartile, third quartile), as appropriate. Categorical variables are presented as counts and percentages.

Short-term repeatability was assessed in the subgroup undergoing reassessment after 24 days. Intraclass correlation coefficients (ICC) and Bland–Altman analyses were used to evaluate agreement between measurements. An ICC ≥ 0.80 was considered indicative of good repeatability. Construct validity was assessed through correlations between DLNO-derived measurements and established indicators of disease severity, including dyspnoea scores, SF-36 domains, arterial blood gases, and the Composite Physiologic Index. Correlation coefficients were interpreted as negligible (< 0.10), weak (0.10–0.29), moderate (0.30–0.49), or strong (≥ 0.50). Construct validity was further explored by comparing DLNO-derived parameters across tertiles of physiological severity defined by PaO_2, alveolar-arterial oxygen gradient ($PA–aO_2$), and CPI. Responsiveness was evaluated by examining associations between 24-week changes in DLNO-derived

measurements and contemporaneous changes in dyspnoea scores, quality of life, physiological indices, and arterial blood gas parameters.

Exploratory analyses evaluated whether 24-week changes in DLNO-derived parameters were associated with subsequent clinical outcomes, including transplant-free survival, mortality, and respiratory hospitalization. Hazard ratios and 95% confidence intervals were estimated using multivariable Cox proportional hazards models adjusted for age, sex, smoking status, ILD subtype, study centre, Charlson comorbidity index, and antifibrotic treatment. The proportional hazards assumption was assessed using Schoenfeld residuals.

Preliminary estimates of clinically meaningful longitudinal change were obtained using both distribution-based and anchor-based approaches. Distribution-based estimates included the standard error of measurement (SEM), 0.2 × standard deviation, and one-third standard deviation. Anchor-based estimates were derived using changes in the SF-36 physical component summary score and Global Rating of Change categories. These analyses were intended to generate clinically interpretable thresholds for future validation studies rather than to establish definitive minimal clinically important differences.

All analyses were performed using observed data without imputation. Statistical analyses were conducted using SPSS software (IBM Corp., Armonk, NY, USA). A two-sided p value < 0.005 was considered statistically significant.

RESULTS

A total of 93 patients were included, comprising 73 individuals with idiopathic pulmonary fibrosis (IPF) and 20 with systemic sclerosis-associated interstitial lung disease (SSc-ILD). Baseline characteristics are summarized in Table 1 and Online Supplementary Tables S1–S2. Participants were predominantly older adults with moderate impairment of gas exchange, mild-to-moderate physiological restriction, and preserved exercise capacity. As expected, patients with SSc-ILD were younger, more frequently female, and received immunomodulatory therapies more often than those with IPF. Across the overall cohort, DLNO-derived measurements were proportionally more impaired than conventional DLCO values, reflecting reductions in both alveolar-capillary membrane conductance (DmCO) and pulmonary capillary blood volume (Vc). No significant differences in DLNO-derived indices were observed between disease subgroups.

Table 1
General characteristics of the study subjects

Males, n (%)	**Overall subjects (n = 93)**	**IPF patients (n = 73)**	**SSc-ILD patients (n = 20)**	**p**
	54 (58)	**54 (74)**	**0**	**< 0.001**
Age, yrs	71 (65–75)	73 (68–76)	61 (55–66)	< 0.001
BMI, Kg/m^2	26.9 ± 4.0	27.8 ± 3.6	23.3 ± 3.3	< 0.001
Smoking history, n (%)	58 (62)	52 (71)	6 (30)	0.001
Dyspnoea level, mMRC	1 (1–2)	1 (0–1)	2 (1–2)	0.002
Hospitalizations since diagnosis, n (%)	21 (23)	11 (15)	10 (50)	0.002
Current treatment				
Corticosteroids, n (%)	17 (18)	1 (1)	16 (80)	< 0.001
Biologic treatments, n (%)	19 (20)	1 (1)	18 (90)	< 0.001
Antifibrotic drugs, n (%)	82 (88)	70 (96)	12 (60)	< 0.001
Mycophenolate, n (%)	14	1	13	< 0.001
Rituximab, n (%)	6	0	6	0.001
Long-term oxygen therapy, n (%)	18 (19)	11 (15)	7 (35)	0.051
PaO_2, mmHg	81 (19–87)	77 (73–82)	86 (77–91)	0.014
PA-aO_2, mmHg	22 (15–25)	25 (21–30)	16 (14–20)	0.004
Lung function tests				
FVC, l	2.68 (2.35–3.44)	2.69 (2.35–3.06)	2.31 (1.98–2.46)	0.001
FVC, % pred.	91 (70–99)	78 (69–92)	78 (68–85)	0.266
FEV_1, l	2.10 (1.89–2.71)	2.25 (1.91–2.59)	1.85 (1.68–1.98)	0.001
FEV_1, % pred.	84 ± 18	86 ± 18	78 ± 19	0.051
FEV_1/FVC	0.82 (0.76–0.86)	0.82 (0.78–0.85)	0.83 (0.78–0.87)	0.246
DLCO, mmol/min/kPa	4.35 ± 1.41	4.5 ± 1.4	3.7 ± 1.2	0.021
DLCO, % pred.	57 ± 16	58 ± 16	51 ± 17	0.094
DLCO/VA, mmol/min/kPa/l	1.21 ± 0.28	1.21 ± 0.29	1.22 ± 0.26	0.942
DLCO/VA, % pred.	91 ± 22	94 ± 22	79 ± 17	0.009

*Values are mean ± standard deviation, median (interquartile range) or number (percentage). Comparisons between groups by t-Student test, Mann-Whitney test of chi-square test.

Abbreviations: IPF, idiopathic pulmonary fibrosis; SSc-ILD, systemic sclerosis–associated interstitial lung disease ; BMI, body mass index; mMRC, modified Medical Research Council; PaO_2, arterial oxygen pressure; PA-aO_2, alveolar-arterial oxygen gradient; FVC, forced vital capacity; FEV_1, forced expiratory volume at 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.

Males, n (%)	Overall subjects (n = 93)	IPF patients (n = 73)	SSc-ILD patients (n = 20)	p
	54 (58)	**54 (74)**	**0**	**< 0.001**
Composite physiological index (CPI)	23.2 ± 9.3	23.9 ± 9.5	20.5 ± 7.9	0.136
6-minutes walked distance, m	447 ± 96	445 ± 100	455 ± 79	0.681
Lung diffusing capacity for nitric oxide				
DLNO, ml/min/mmHg	53.7 ± 17.0	54.9 ± 17.0	48.8 ± 16.8	0.167
DLNO, % pred.	47 ± 14	47 ± 14	49 ± 16	0.645
DLNO/VA, ml/min/mmHg/l	14.4 ± 2.9	14.4 ± 2.9	14.3 ± 2.9	0.943
DLNO/VA, % pred.	73 ± 15	74 ± 14	68 ± 14	0.069
DmCO, ml/min/mmHg	46 (33–56)	44 (34–56)	38 (28–47)	0.234
DmCO, % pred.	41 ± 17	41 ± 16	44 ± 19	0.383
Vc, ml	31 (28–34)	33 (28–41)	33 (26–38)	0.791
Vc, % pred.	54 (48–58)	56 (46–66)	53 (44–68)	0.836
DmCO/VA, ml/min/mmHg/l	12 (9–14)	13 (10–14)	10 (7–11)	0.534
DmCO/VA, % pred.	68 ± 20	69 ± 20	66 ± 20	0.491
Vc/VA, ml/l	76 (68–98)	8.9 (7.0–11.0)	10.4 (8.7–11.6)	0.245
Vc/VA, % pred.	76 (68–98)	90 (73–109)	87 (71–102)	0.472

*Values are mean ± standard deviation, median (interquartile range) or number (percentage). Comparisons between groups by t-Student test, Mann-Whitney test of chi-square test.

Abbreviations: IPF, idiopathic pulmonary fibrosis; SSc-ILD, systemic sclerosis–associated interstitial lung disease ; BMI, body mass index; mMRC, modified Medical Research Council; PaO_2, arterial oxygen pressure; PA-aO_2, alveolar-arterial oxygen gradient; FVC, forced vital capacity; FEV_1, forced expiratory volume at 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.

Repeatability

Short-term repeatability was assessed in a randomly selected subgroup of 40 participants who underwent repeat testing after 24 days. DLNO-derived measurements demonstrated good to excellent repeatability. Intraclass correlation coefficients exceeded 0.80 for DLNO, DmCO, and Vc in the overall cohort and in both disease subgroups (Table 2). Similar repeatability was observed across age, sex, and BMI categories. Bland–Altman analyses showed acceptable agreement and no evidence of systematic measurement bias (Online Supplementary Figure s1).

Table 2

Reliability analysis. Intraclass correlation coefficients between percent-predicted values at baseline and 24-day visits*

	Overall group		Patients with IPF		Patients with SSc-ILD	
	ICC (95%CI)	**p-value**	**ICC (95%CI)**	**p-value**	**ICC (95%CI)**	**p-value**
FVC, % pred.	0.924 (0.962 – 0.959)	< 0.001	0.925 (0.882– 0.953)	< 0.001	0.967 (0.915– 0.988)	< 0.001
DLCO, % pred.	0.875 (0.814– 0.956)	< 0.001	0.867 (0.794– 0.916)	< 0.001	0.900 (0.754– 0.961)	< 0.001
DLCO/VA, % pred.	0.903 (0.855– 0.935)	< 0.001	0.899 (0.843– 0.937)	< 0.001	0.860 (0.666– 0.945)	< 0.001
DLNO, % pred.	0.810 (0.722– 0.873)	< 0.001	0.817 (0.769– 0.930)	< 0.001	0.924 (0.796– 0.973)	< 0.001
DLNO/VA, % pred.	0.926 (0.888– 0.951)	< 0.001	0.929 (0.887– 0.955)	< 0.001	0.896 (0.728– 0.962)	< 0.001
DmCO, % pred.	0.819 (0.721– 0.883)	< 0.001	0.809 (0.691– 0.882)	< 0.001	0.809 (0.681– 0.926)	< 0.001
DmCO/VA, % pred.	0.764 (0.657– 0.840)	< 0.001	0.786 (0.675– 0.862)	< 0.001	0.613 (0.186– 0.845)	0.004
Vc, % pred.	0.886 (0.751- 0956)	< 0.001	0.850 (0.789– 0.907)	< 0.001	0.882 (0.696– 0.957)	< 0.001
Vc/VA, % pred.	0.837 (0.743– 0.898)	< 0.001	0.824 (0.765– 0.886)	< 0.001	0.815 (0.723– 0.894)	< 0.001

*Values are intraclass correlation coefficients and 95% confidence intervals.

Abbreviations: IPF, idiopathic pulmonary fibrosis; SSc-ILD, systemic sclerosis–associated interstitial lung disease; FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.

Construct validity

DLNO-derived measurements showed significant associations with established physiological indicators of disease severity. Correlations with dyspnoea scores and arterial blood gas measurements were generally weak to moderate, whereas stronger associations were observed with the Composite Physiologic Index (CPI), particularly for DLNO and DLNO/VA (Table 3). Associations with health-related quality of life were limited. Additional support for construct validity was provided by analyses across tertiles of physiological severity. Patients with lower PaO_2, higher alveolar-arterial oxygen gradients, or worse CPI values consistently exhibited lower DLNO, DmCO, and Vc measurements (Table 4). Similar patterns were observed within the individual IPF and SSc-ILD subgroups (Online Supplementary Tables S3–S4).

Table 3

Validity analysis. Correlation between percent-predicted values and other measures in all study subjects*

Dyspnea level, mMRC	FVC, % pred.	DLCO, % pred.	DLNO, % pred.	DLNO/VA, % pred.	DmCO, % pred.	DmCO/VA, % pred.	Vc, % pred.	Vc/VA, % pred.
	ρ=-0.148; p = 0.157	**ρ=-0.328; p = 0.001**	**ρ=-0.194; p = 0.064**	**ρ=-0.262; p = 0.012**	**ρ=-0.169; p = 0.108**	**ρ=-0.183; p = 0.081**	**ρ=-0.156; p = 0.137**	**ρ=-0.079; p = 0.455**
BDI/TDI task	**ρ=-0.311; p = 0.002**	**ρ=-0.422; p < 0.001**	**ρ=-0.322; p = 0.002**	**ρ=-0.301; p = 0.004**	**ρ=-0.264; p = 0.011**	**ρ=-0.229; p = 0.028**	**ρ=-0.240; p = 0.021**	ρ=-0.087; p = 0.408
BDI/TDI functional	**ρ=-0.348; p = 0.001**	**ρ=-0.426; p < 0.001**	**ρ=-0.331; p = 0.001**	**ρ=-0.288; p = 0.005**	**ρ=-0.316; p = 0.002**	**ρ=-0.290; p = 0.005**	ρ=-0.185; p = 0.077	ρ = 0.007; p = 0.947
BDI/TDI effort	**ρ=-0.332; p = 0.001**	**ρ=-0.455; p < 0.001**	**ρ=-0.358; p < 0.001**	**ρ=-0.358; p < 0.001**	**ρ=-0.327; p = 0.001**	**ρ=-0.285; p = 0.006**	**ρ=-0.228; p = 0.029**	ρ=-0.054; p = 0.611
SF36-PCS	ρ = 0118.; p = 0.338	ρ = 0.228; p = 0.062	ρ = 0.091; p = 0.461	ρ = 0.193; p = 0.115	ρ = 0.113; p = 0.359	ρ = 0.121; p = 0.324	ρ=-0.007; p = 0.955	ρ=-0.049; p = 0.694
SF36-MCS	ρ=-0.092; p = 0.457	ρ=-0.197; p = 0.107	ρ=-0.016; p = 0.895	ρ=-0.107; p = 0.383	ρ=-0.043; p = 0.725	ρ=-0.056; p = 0.653	ρ = 0.010; p = 0.938	ρ = 0.053; p = 0.665
CPI	**ρ = 0.163; p = 0.118**	**ρ = 0.939; p < 0.001**	**ρ = 0.616; p < 0.001**	**ρ = 0.807; p < 0.001**	**ρ = 0.356; p < 0.001**	**ρ = 0.351; p = 0.001**	**ρ = 0.767; p < 0.001**	**ρ = 0.607; p < 0.001**
PaO_2, mmHg	**ρ = 0.362; p < 0.001**	**ρ = 0.346; p = 0.001**	**ρ = 0.530; p < 0.001**	**ρ = 0.279; p = 0.007**	**ρ = 0.439; p < 0.001**	**ρ = 0.250; p = 0.017**	**ρ = 0.295; p = 0.005**	ρ = 0.047; p = 0.660
PA-aO_2, mmHg	**ρ=-0.228; p = 0.029**	**ρ=-0.311; p = 0.003**	**ρ=-0.451; p < 0.001**	**ρ=-0.316; p = 0.002**	**ρ=-0.344; p = 0.001**	ρ=-0.195; p = 0.064	**ρ=-0.246; p = 0.019**	ρ=-0.091; p = 0.392

*Values are Spearman correlation coefficients (ρ) and p-values. Significant correlations are highlighted in bold.

Abbreviations: FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume; mMRC, modified Medical Research Council; BDI/TDI, baseline and transition dyspnoea indexes; SF36, 36-Item Short Form Health Survey; PCS, physical component summary; MCS, mental component summary; CPI, composite physiologic index; PaO_2, arterial oxygen pressure; PA-aO_2, alveolar-arterial oxygen gradient.

Table 4
Validity analysis. Mean (SD) percent-predicted values by tertiles of other measures in all study subjects*

	DLNO, % pred.	DLNO/VA, % pred.	DmCO, % pred.	DmCO/VA, % pred.	Vc, % pred.	Vc/VA, % pred.
PaO_2, mmHg						
< 74.0	38 ± 12	66 ± 15	33 ± 13	61 ± 17	53 ± 22	96 ± 48
74.0-82.3	49 ± 11	77 ± 12	42 ± 12	70 ± 18	67 ± 23	104 ± 36
> 82.3	54 ± 15	76 ± 15	49 ± 20	74 ± 22	67 ± 19	102 ± 34
p-value	< 0.001	0.007	0.001	0.040	0.021	0.703
PA-aO_2, mmHg						
< 19.7	52 ± 13	77 ± 14	46 ± 17	73 ± 20	63 ± 17	99 ± 34
19.7–26.2	50 ± 14	76 ± 13	43 ± 16	70 ± 20	68 ± 24	104 ± 33
> 26.2	38 ± 12	65 ± 14	34 ± 13	61 ± 18	54 ± 24	97 ± 52
p-value	< 0.001	0.003	0.007	0.057	0.066	0.773
Composite physiological index						
< 18.95	37 ± 12	59 ± 12	35 ± 14	59 ± 20	45 ± 14	79 ± 34
18.95–26.63	48 ± 11	75 ± 9	43 ± 15	73 ± 19	59 ± 18	96 ± 39
> 26.63	56 ± 14	84 ± 9	46 ± 19	73 ± 19	80 ± 19	124 ± 33
p-value	< 0.001	< 0.001	0.016	0.010	< 0.001	< 0.001

*Values are mean ± standard deviation. Comparisons by ANOVA.

Abbreviations: PaO_2, arterial oxygen pressure; PA-aO_2, alveolar-arterial oxygen gradient; DLNO, diffusing capacity of the lung for nitric oxide; VA, alveolar volume; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.

Responsiveness to longitudinal change

Over the 24-week observation period, DLNO-derived measurements demonstrated measurable longitudinal changes. DLNO declined significantly more than DLCO (− 0.5 [− 4.7, 0.7] vs. 0 [− 5.5, 4.0] % predicted; p = 0.020) (Online Supplementary Table S5). Changes in DLNO-derived parameters were modestly associated with changes in dyspnoea scores, particularly mMRC, whereas associations with changes in quality of life, CPI, and arterial blood gases were generally weak or absent (Online Supplementary Table S6). These findings support the responsiveness of DLNO-derived measurements to short-term physiological changes in fibrotic ILD.

Associations with clinical outcomes

Several DLNO-derived parameters were associated with transplant-free survival, mortality, and respiratory hospitalization in adjusted models evaluating each parameter separately (Tables 5, 6, and Online Supplementary Table S7). However, when all DLNO-derived measurements were analysed simultaneously in the same multivariable models, only DmCO decline retained independent associations with transplant-free survival (HR 0.949, 95% CI 0.925–0.973; p < 0.001), 1-year mortality (HR 1.183, 95% CI 1.069–1.308; p < 0.001), 2-year mortality (HR 1.235, 95% CI 1.079–1.417; p = 0.002), and

respiratory hospitalization (HR 1.174, 95% CI 1.054–1.308; p = 0.001). These findings should be interpreted as exploratory given the limited number of events.

Table 5
Prognostic value of 24-week changes in pulmonary function parameters for 2-year transplant-free survival*

FVC decrease, % pred.	**HR (95%CI)†**	**P**
	0.909 (0.818–1.010)	**0.076**
DLCO decrease, % pred.	1.036 (0.951–1.127)	0.419
DLNO decrease, % pred.	0.936 (0.906–0.967)	< 0.001
DLNO/VA decrease, % pred.	0.814 (0.734–0.903)	< 0.001
DmCO decrease, % pred.	0.949 (0.925–0.973)	< 0.001
DmCO/VA decrease, % pred.	0.922 (0.888–0.958)	< 0.001
Vc decrease, % pred.	0.997 (0.994-1.000)	0.037
Vc/VA decrease, % pred.	0.999 (0.997-1.000)	0.035
*Values are hazard ratios (HR) and 95% confidence intervals (95%CI).		
†Multivariable Cox models adjusted for age, sex, smoking status, comorbidities, disease subtype and treatment.		
Abbreviations: FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.		

Table 6
Predictive value of pulmonary function changes for 1- and 2-year mortality *

	Mortality at 1 year		**Mortality at 2 years**	
	HR (95%CI)†	**P**	**HR (95%CI)†**	**P**
FVC decrease, % pred.	1.126 (0.962–1.316)	0.139	1.068 (0.931–1.226)	0.349
DLCO decrease, % pred.	1.045 (0.920–1.187)	0.499	1.029 (0.922–1.148)	0.608
DLNO decrease, % pred.	1.081 (1.041–1.123)	< 0.001	1.077 (1.037–1.118)	< 0.001
DLNO/VA decrease, % pred.	1.343 (1.106–1.631)	0.003	1.382 (1.146–1.667)	0.001
DmCO decrease, % pred.	1.065 (1.032–1.099)	< 0.001	1.062 (1.030–1.094)	< 0.001
DmCO/VA decrease, % pred.	1.102 (1.045–1.162)	< 0.001	1.102 (1.048–1.159)	< 0.001
Vc decrease, % pred.	1.004 (1.001–1.007)	0.008	1.003 (1.000-1.006)	0.023
Vc/VA decrease, % pred.	1.002 (1.000-1.003)	0.008	1.001 (1.000-1.003)	0.022
*Values are hazard ratios (HR) and 95% confidence intervals (95%CI).				
†Multivariable Cox models adjusted for age, sex, smoking status, comorbidities, disease subtype and treatment.				
Abbreviations: FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume; DLNO, diffusing capacity of the lung for nitric oxide; DmCO, alveolar-capillary membrane conductance; Vc, pulmonary capillary blood volume.				

Preliminary estimates of clinically meaningful change

Distribution-based and anchor-based approaches yielded broadly consistent estimates of clinically meaningful longitudinal change for DLNO-derived measurements (Online Supplementary Tables S8–S9). Estimated thresholds for meaningful change (% predicted) were approximately 3.1 for DLNO, 4.9 for DmCO, and 7.3 for Vc (Fig. 1). These estimates were supported by similar results across alternative calculation methods and by the expected gradient across Global Rating of Change categories.

In exploratory analyses, participants exhibiting larger declines in DmCO showed a higher incidence of adverse clinical outcomes during follow-up (Fig. 2). These findings support the potential clinical interpretability of longitudinal changes in DmCO, although external validation is required before adoption in clinical practice.

DISCUSSION

This prospective multicentre study provides a comprehensive evaluation of DLNO-derived measurements in patients with fibrotic interstitial lung disease, including idiopathic pulmonary fibrosis (IPF) and systemic sclerosis-associated ILD (SSc-ILD). Several findings deserve consideration. First, DLNO-derived measurements demonstrated good short-term repeatability across both disease entities. Second, these parameters showed consistent associations with established physiological indicators of disease severity, supporting their construct validity. Third, DLNO-derived measurements were responsive to longitudinal changes over a 24-week period. Finally, changes in DmCO were associated with subsequent clinical outcomes, suggesting potential clinical relevance that warrants further investigation.

The physiological basis supporting the use of DLNO-derived measurements in fibrotic lung disease is well established. Unlike DLCO, which reflects the combined effects of alveolar-capillary membrane conductance and pulmonary capillary blood volume, the simultaneous measurement of DLNO and DLCO allows partitioning of pulmonary diffusing capacity into DmCO and Vc according to the Roughton–Forster model [28–30]. This approach may provide a more detailed characterization of the mechanisms underlying gas-exchange impairment in ILD, where membrane thickening, vascular remodelling, capillary loss, and ventilation-perfusion abnormalities frequently coexist [31, 32].

In the present study, DLNO was proportionally more impaired than DLCO, reflecting reductions in both DmCO and Vc. These observations are consistent with previous reports demonstrating that DLNO may be particularly sensitive to abnormalities affecting the alveolar-capillary membrane [28–30]. At the same time, the concomitant reduction in Vc supports the concept that vascular involvement contributes substantially to physiological impairment in fibrotic ILD, particularly in conditions such as SSc-ILD where pulmonary vasculopathy is a recognized component of disease pathogenesis [31, 32]. Although IPF and SSc-ILD represent biologically distinct entities, both ultimately affect the integrity of the alveolar-capillary unit. Their inclusion in the present study therefore allowed evaluation of DLNO-derived measurements across two complementary fibrotic ILD phenotypes characterized by different patterns of membrane and vascular involvement.

One of the most robust findings of the study was the excellent repeatability of DLNO-derived measurements. Intraclass correlation coefficients exceeded 0.80 for the principal variables of interest, indicating reproducibility comparable to that reported for conventional pulmonary function measurements [5, 33]. Reliable physiological measurements are essential for both clinical monitoring and research applications. The repeatability observed in the present study supports the technical robustness of DLNO-derived parameters and suggests that these measurements may be suitable for longitudinal assessment in patients with fibrotic ILD.

Construct validity was further supported by the observed associations between DLNO-derived measurements and established physiological indicators of disease severity. DLNO, DmCO and, to a lesser extent, Vc demonstrated significant associations with arterial oxygenation, alveolar-arterial oxygen gradients, and the Composite Physiologic Index. These findings are biologically plausible because impairment of membrane conductance and pulmonary capillary recruitment

would be expected to contribute directly to abnormalities in gas exchange. By contrast, correlations with patient-reported outcomes were generally modest. This observation is not unexpected, as symptoms and health-related quality of life in ILD are influenced by multiple factors beyond pulmonary physiology, including cardiovascular comorbidity, physical conditioning, psychological status, and extrapulmonary disease manifestations. Consequently, physiological measurements and patient-reported outcomes should be regarded as complementary rather than interchangeable dimensions of disease assessment.

An additional finding of interest was the responsiveness of DLNO-derived measurements to longitudinal change. Over the 24-week observation period, DLNO declined more markedly than conventional DLCO. Although the associations between changes in DLNO-derived measurements and changes in symptoms or quality of life were modest, these findings suggest that partitioning gas-transfer abnormalities into membrane and vascular components may provide useful information regarding short-term physiological evolution. Previous studies have highlighted the importance of detecting relatively small changes in physiological parameters in progressive fibrotic lung disease, and the present findings support further evaluation of DLNO-derived measurements in this context [5, 33].

The exploratory analyses evaluating clinical outcomes also yielded noteworthy observations. Changes in DmCO showed the most consistent associations with transplant-free survival, mortality, and respiratory hospitalization. These findings are biologically plausible because DmCO primarily reflects the integrity of the alveolar-capillary membrane, which is directly affected by fibrosis-related injury and remodelling. Similar observations have been reported in previous studies linking gas-transfer abnormalities, exercise-induced hypoxemia, and physiological progression with adverse outcomes in fibrotic lung diseases [34–39].

However, these findings should be interpreted with caution. The study was not specifically powered to evaluate prognostic performance, and the number of mortality and transplant events was limited. Consequently, the observed associations should be considered hypothesis-generating rather than definitive evidence of prognostic superiority over conventional physiological indices. Larger studies with external validation cohorts will be required to determine whether DmCO provides incremental prognostic information beyond established markers such as FVC and DLCO [34–39].

The present study also provides preliminary estimates of clinically meaningful longitudinal change for DLNO-derived measurements. Distribution-based and anchor-based approaches yielded relatively consistent thresholds, suggesting that changes of approximately 3% predicted for DLNO, 5% predicted for DmCO, and 7% predicted for Vc may represent clinically interpretable changes. These estimates should nevertheless be regarded as exploratory. Unlike FVC and DLCO, for which clinically meaningful thresholds have been explored in larger populations [5, 40], evidence supporting clinically relevant change thresholds for DLNO-derived measurements remains limited. Furthermore, the anchor-based analyses relied on a generic health-related quality-of-life instrument that demonstrated only modest correlations with physiological variables. Consequently, these thresholds should primarily be viewed as a framework for future validation studies rather than definitive minimal clinically important differences.

Several limitations should be acknowledged. First, although the sample size was adequate for the primary objectives related to repeatability, validity, and responsiveness, it was relatively small for outcome analyses. The limited number of events restricts the stability of multivariable estimates and precludes firm conclusions regarding prognostic performance. Second, the inclusion of both IPF and SSc-ILD introduced biological and clinical heterogeneity. Although this design allowed evaluation across two distinct fibrotic ILD phenotypes, the study was not powered to establish disease-specific prognostic models. Third, the follow-up period was relatively short for a chronic progressive disease such as fibrotic ILD. Fourth, recruitment from tertiary referral centres may limit generalizability to broader clinical populations. Finally, residual confounding related to comorbidities, treatment changes, pulmonary hypertension, or unmeasured disease characteristics cannot be completely excluded despite statistical adjustment.

The study also has important strengths. To our knowledge, it represents one of the largest prospective evaluations of DLNO-derived measurements in fibrotic ILD. Standardized physiological assessments were performed in a dedicated reference laboratory, longitudinal follow-up was available for all participants, and multiple complementary dimensions of measurement performance—including repeatability, construct validity, responsiveness, and clinical interpretability—were evaluated simultaneously. In addition, the inclusion of both IPF and SSc-ILD provided the opportunity to examine DLNO-derived measurements across two clinically relevant fibrotic ILD phenotypes with distinct pathobiological characteristics.

In conclusion, DLNO-derived measurements, particularly DmCO, demonstrated good reproducibility, physiological validity, and responsiveness to longitudinal change in patients with IPF and SSc-ILD. Associations between DmCO decline and subsequent clinical outcomes suggest potential clinical relevance, although confirmation in larger prospective cohorts is required. Together, these findings support the role of DLNO-derived measurements as complementary physiological tools for the assessment and longitudinal monitoring of fibrotic interstitial lung disease.

Abbreviations

ATS – American Thoracic Society

BDI – Baseline Dyspnea Index

BMI – Body Mass Index

CI – Confidence Interval

CO – Carbon Monoxide

CPI – Composite Physiologic Index

DLCO – Diffusing Capacity of the Lung for Carbon Monoxide

DLNO – Diffusing Capacity of the Lung for Nitric Oxide

DmCO – Alveolar-Capillary Membrane Conductance for Carbon Monoxide

ERS – European Respiratory Society

FVC – Forced Vital Capacity

GRC – Global Rating of Change

HR – Hazard Ratio

HRCT – High-Resolution Computed Tomography

ICC – Intraclass Correlation Coefficient

ILD – Interstitial Lung Disease

IPF – Idiopathic Pulmonary Fibrosis

IQR – Interquartile Range

MCID – Minimal Clinically Important Difference

mMRC – Modified Medical Research Council

NO – Nitric Oxide

PA-aO_2 – Alveolar-Arterial Oxygen Gradient

PaO_2 – Arterial Partial Pressure of Oxygen

SD – Standard Deviation

SEM – Standard Error of Measurement

SF-36 – 36-Item Short Form Health Survey

SSc-ILD – Systemic Sclerosis–Associated Interstitial Lung Disease

TDI – Transition Dyspnea Index

VA – Alveolar Volume

Vc – Pulmonary Capillary Blood Volume

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Hospital Universitario La Paz (PI-4818), and all participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

Data are available upon reasonable request. The datasets used and/or analysed during the current study are available for the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This study was supported by grants from Sociedad Madrileña de Neumología y Cirugía Torácica (NEUMOMADRID 2022) and from Sociedad Española de Neumología y Cirugía Torácica (Beca SEPAR 1599) to A Tenes; and from Instituto de Salud Carlos III (PI22-01262, PI25-00892 and DTS24/00097) to F García-Río.

Author's contribution

F.G.-R. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. A.T., and F.G.-R. were responsible for developing the research question. A.T., J.R.B., A.J.-O., P.M., L.G.C., C.M.L., M.T.-V., E.M.-C., R.G., R.C., and F.G.-R. were responsible for the study design and collection of data. A.T., M.T.-V., and F.G.-R. were responsible for study management and coordination. A.T., M.T.-V., E.M.-C., R.G., R.C., and F.G.-R.

were responsible for the analysis. F.G.-R. drafted the paper. All authors have read and approved the final manuscript. All authors had final responsibility for the decision to submit for publication.

Acknowledgements

Not applicable

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Figures

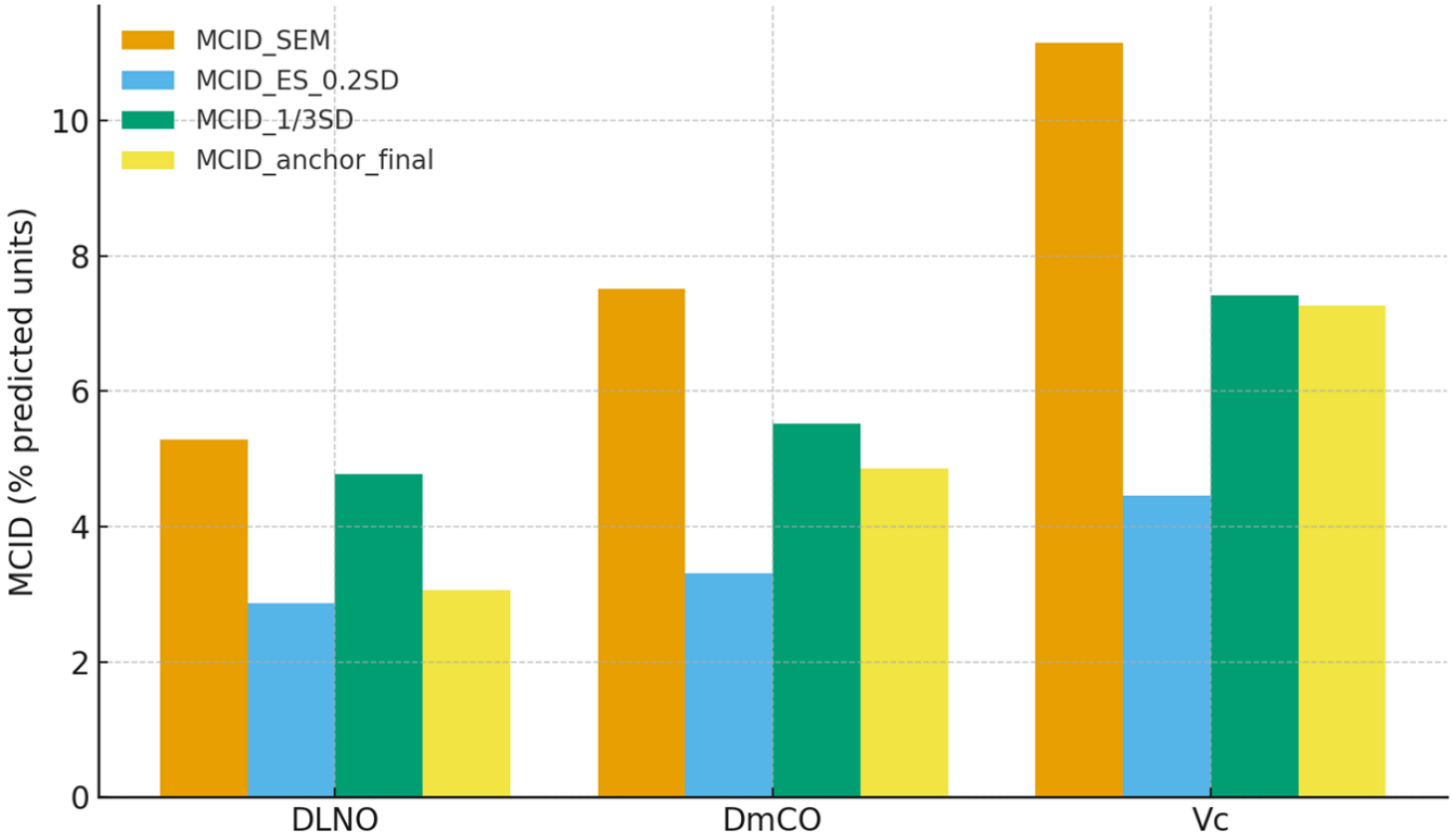


Figure 1

Estimated minimal clinically important difference (MCID) values for DLNO, DmCO, and Vc according to four methods: standard error of measurement (SEM), 0.2 × standard deviation (SD), 1/3 × SD, and anchor-based approach.

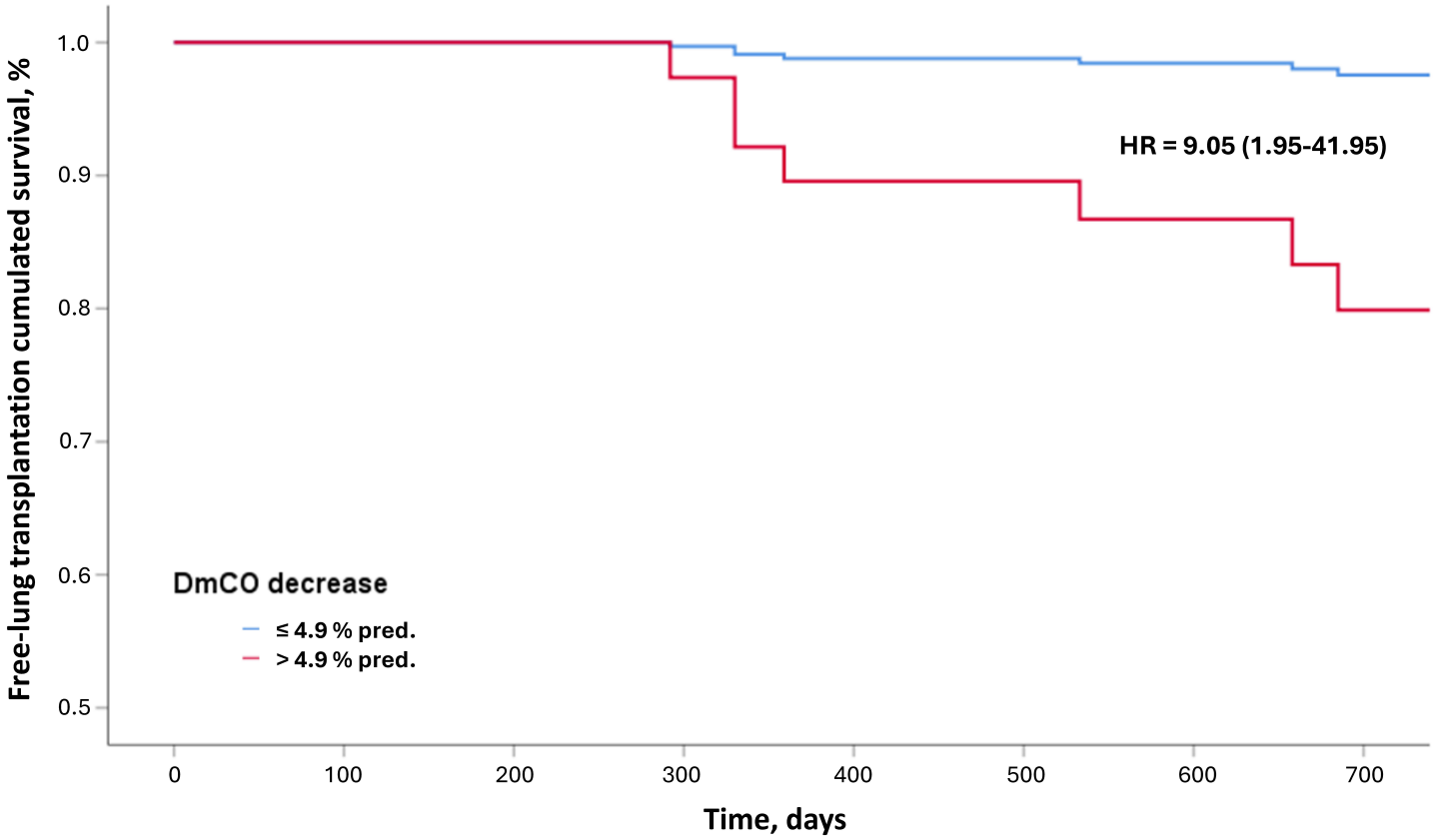


Figure 2

Two-year transplant-free survival stratified by 24-week DmCO decrease above or below the minimal clinically important difference (MCID; 4.9% predicted).

Supplementary Files

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- SupplementalMaterial.docx