Combined measurement of carbon monoxide and nitric oxide lung transfer does not improve the identification of pulmonary hypertension in systemic sclerosis

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Compared with *T*LCO alone, combined *T*LCO–*T*LNO measurement does not improve detection of PH in unselected SSc patients http://ow.ly/ITHO30eldMk

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ABSTRACT Screening is important to determine whether patients with systemic sclerosis (SSc) have pulmonary hypertension because earlier pulmonary hypertension treatment can improve survival in these patients. Although decreased transfer factor of the lung for carbon monoxide (*T*LCO) is currently considered the best pulmonary function test for screening for pulmonary hypertension in SSc, small series have suggested that partitioning *T*LCO into membrane conductance (diffusing capacity) for carbon monoxide (*D*MCO) and alveolar capillary blood volume (*V*C) through combined measurement of *T*LCO and transfer factor of the lung for nitric oxide (*T*LNO) is more effective to identify pulmonary hypertension in SSc patients compared with *T*LCO alone. Here, the objective was to determine whether combined *T*LCO–*T*LNO partitioned with recently refined equations could more accurately detect pulmonary hypertension than *T*LCO alone in SSc.

For that purpose, 572 unselected consecutive SSc patients were retrospectively recruited in seven French centres.

Pulmonary hypertension was diagnosed with right heart catheterisation in 58 patients. *T*LCO, *T*LNO and *V*C were all lower in SSc patients with pulmonary hypertension than in SSc patients without pulmonary hypertension. The area under the receiver operating characteristic curve for the presence of pulmonary hypertension was equivalent for *T*LCO (0.82, 95% CI 0.79–0.85) and *T*LNO (0.80, 95% CI 0.76–0.83), but lower for *V*C (0.75, 95% CI 0.71–0.78) and *D*MCO (0.66, 95% CI 0.62–0.70).

Compared with *T*LCO alone, combined *T*LCO–*T*LNO does not add capability to detect pulmonary hypertension in unselected SSc patients.

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterised by widespread vasculopathy and excessive fibrosis in multiple organs, including the skin, digestive tract, kidneys, heart and lungs [1]. Since the early 1980s, with the advent of angiotensin-converting enzyme inhibitors that have improved the treatment of scleroderma renal crisis, lung diseases have become the leading cause of death in SSc [2]. Interstitial lung disease (ILD) and pulmonary hypertension are the two main forms of pulmonary involvement in SSc [3]. Interstitial abnormalities on high-resolution computed tomography (HRCT) are found in many scleroderma patients, but lesions are generally of limited extent [4], and early deaths attributable to ILD are uncommon in SSc [5, 6]. Although less frequent than ILD, pulmonary hypertension either isolated or associated with ILD has a poor prognosis in SSc [3]. Early diagnosis and prompt therapy of pulmonary hypertension are beneficial from a prognostic standpoint in SSc patients either with or without ILD [7, 8], and recommendations for active screening of pulmonary hypertension in SSc have therefore been made [9].

Several pulmonary function tests are widely used for screening of pulmonary hypertension. Among them, impaired transfer factor of the lung for carbon monoxide (T_{LCO}) has been recognised as the most sensitive marker of pulmonary hypertension in SSc [10, 11]. Nevertheless, this test lacks specificity, at least in part because ILD contributes to altering T_{LCO} [12]. In order to overcome this lack of specificity, a "partition" of T_{LCO} into two transfer components, *i.e.* membrane conductance (diffusing capacity) for carbon monoxide (D_{MCO}) and alveolar capillary blood volume (V_C), has been proposed [13]. A decreased D_{MCO} is interpreted as a "thickening" of the alveolo-capillary membrane or as a decrease in lung area, while a decreased V_C is considered to represent reduced blood volume in the ventilated alveoli [14]. Partitioning T_{LCO} can be calculated with the Roughton–Forster equation either by measuring T_{LCO} at several oxygenation levels or by combining measurement of T_{LCO} with transfer factor of the lung for nitric oxide (T_{LNO}) [15–17]. Several studies have evaluated the diagnostic power of D_{MCO} and V_C to identify pulmonary hypertension in SSc patients. Yet, to date, only small, single-centre series of SSc patients have been studied and results have been predominantly contradictory [18–20]. In addition, the equations used for the calculation of D_{MCO} and V_C with combined T_{LCO}–T_{LNO} in these studies are currently viewed as inappropriate [16, 21].

Therefore, in a large, multicentre sample of unselected patients with SSc who had combined T_{LCO}–T_{LNO} measurements, we aimed to assess the usefulness of the T_{LCO} partition into D_{MCO} and V_C for the identification of pulmonary hypertension, with D_{MCO} and V_C being calculated according to the latest equations proposed by the European Respiratory Society (ERS) Task Force [16]. We also aimed to study whether or not calculation of D_{MCO} and V_C helps to predict overall survival in patients with SSc.

Methods

Patients

Consecutive unselected patients with SSc were retrospectively recruited in seven French medical centres (Besançon, Bordeaux, Paris-Cochin, Lille, Nantes, Poitiers and Rouen). Patients were evaluated in stable conditions. The diagnosis of SSc was made or confirmed by a specialist according to current recommendations [22]. Inclusion date was the date of measurement of combined T_{LCO}–T_{LNO}. Clinical information was retrospectively collected for all participating patients, and included cutaneous disease subset (according to the latest recommendations [22]), disease duration (from the date of first disease manifestation other than Raynaud's phenomenon) and history of digital ulceration. Biochemical and serological blood tests, including haemoglobin, antinuclear antibodies, anticentromere antibodies and antitopoisomerase I antibodies, were also collected. ILD was assessed by the presence of subpleural

ground-glass opacities and/or interstitial reticular pattern with or without traction bronchiectasis, and/or honeycomb cysts on HRCT [4, 20].

The study was approved by the Institutional Review Board of the French Language Society for Respiratory Medicine (Société de Pneumologie de Langue Française), which deemed it to be observational and therefore waived the need for informed consent (CEPRO 2015-025).

Assessment of pulmonary hypertension

All patients underwent Doppler echocardiography at the time of pulmonary function tests, for the measurement of left ventricular ejection fraction and tricuspid regurgitant jet velocity. Right heart catheterisation was performed in patients with tricuspid regurgitant jet velocity >3 $m \cdot s^{-1}$ and in patients with unexplained dyspnoea along with tricuspid regurgitant jet velocity >2.5 $m \cdot s^{-1}$ [11]. A diagnosis of pulmonary hypertension was retained in patients with a mean pulmonary artery pressure $\geqslant$25 mmHg together with a mean pulmonary arterial wedge pressure $\leqslant$15 mmHg measured during right heart catheterisation performed at rest [7].

Pulmonary function tests

Forced expiratory flow in 1 s, forced vital capacity, slow vital capacity, functional residual capacity, residual volume and total lung capacity were assessed with standard pulmonary function test equipment in each participating centre according to the ERS guidelines [23, 24]. References values for spirometry and lung volumes were from Quanjer *et al.* [25].

Combined *T*LCO–*T*LNO measurements were performed with a commercially available device following daily calibration (Medisoft, Dinant, Belgium), according to a procedure described elsewhere [14]. Calculations of *D*MCO and *V*C were made according to the most recent recommendations (see supplementary material) [16]. Reference values for *T*LCO, *T*LNO, *V*C and *D*MCO were from Zavorsky *et al.* [16].

Statistical analyses

Patients were first divided into four groups according to the results of ILD and pulmonary hypertension assessments: SSc patients without pulmonary involvement (Group A), SSc patients with ILD alone (Group B), SSc patients with both ILD and pulmonary hypertension (Group C), and SSc patients with pulmonary hypertension alone (Group D). Data are presented as mean±SD for quantitative variables and as percentages for categorical variables. Comparisons of categorical data between groups were performed using Fisher's exact test. ANOVA was performed to evaluate differences of quantitative variables between the four groups. Scheffe's tests were applied to evaluate pairwise comparisons when a significant difference was found by ANOVA. The Kaplan–Meier method was used to illustrate survival according to the group, with statistically significant differences in event rates assessed *via* the log-rank test.

Analyses were then performed between SSc patients without pulmonary hypertension (Groups A and B) and SSc patients with pulmonary hypertension (Groups C and D). The area under the receiver operating characteristic (ROC) curve (AUC) was used to assess the ability of *T*LCO, *T*LNO, *V*C, *D*MCO, *T*LNO/*T*LCO and *D*MCO/*V*C to discriminate between the presence and absence of pulmonary hypertension. The optimal threshold for the identification of pulmonary hypertension was assessed according to the Youden index of ROC curve analysis in order to maximise both sensitivity and specificity [26]. Comparisons of AUC values were performed with MedCalc Statistical Software version 16.8.4 (MedCalc, Ostend, Belgium). Univariate analysis based on the proportional hazards model was used to examine the relationship between survival and *T*LCO, *T*LNO, *D*MCO, *V*C and relevant clinical data. Multivariate analysis using Cox's proportional hazards regression model was used to examine the independent effect of each of these variables on survival. Variables with $p<0.20$ in the univariate analysis were included in the multivariate model.

A p-value of 0.05 was deemed to be statistically significant. All analyses except comparisons of AUC values were performed with SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

We identified a total of 572 SSc patients who had combined *T*LCO–*T*LNO measurements between November 2006 and April 2016 in the seven participating centres. The main characteristics of the four groups of patients, divided according to the results of pulmonary hypertension and of ILD assessments, are given in table 1.

Pulmonary function tests

*T*LCO and *T*LNO were the lowest in SSc patients with pulmonary hypertension (Groups C and D), and were also lower in SSc patients with ILD (Group B) than in SSc patients without any pulmonary involvement

TABLE 1 Main characteristics at inclusion of patients with systemic sclerosis (SSc), divided into four groups: SSc patients without any pulmonary involvement, *i.e.* neither interstitial lung disease (ILD) nor pulmonary hypertension (Group A), SSc patients with ILD but without pulmonary hypertension (Group B), SSc patients with both ILD and pulmonary hypertension (Group C), and SSc patients without ILD but with pulmonary hypertension (Group D)

	Group A	Group B	Group C	Group D	p-value
Subjects	313	201	35	23	
Age years	56±14[¶,+]	58±13[+]	63±11	67±10	<0.0001
BMI kg·m^{-2}	25±5	24±4	26±5	24±5	0.33
Female	268 (86)[#,¶]	148 (74)	21 (60)	20 (87)	0.0001
Ever-smoker	113 (36)	70 (35)	10 (29)	5 (22)	0.72
Cutaneous subtype					<0.0001
Limited	277 (88)[#,¶]	106 (53)[+]	17 (49)[+]	21 (91)	
Diffuse	36 (12)[#,¶]	95 (47)[+]	18 (51)[+]	2 (9)	
Antinuclear antibodies >1/160	295 (94)	190 (95)	33 (94)	22 (96)	0.81
Antitopoisomerase I antibodies	33 (11)[#,¶]	70 (35)[+]	9 (26)	3 (13)	0.006
Anticentromere antibodies	203 (65)[#,¶]	45 (22)[+]	9 (26)[+]	12 (52)	<0.0001
Disease duration before T0 months	92±92[+]	102±93[+]	136±104	166±142	0.0013
History of digital ulcers	95 (30)[¶]	81 (40)	20 (57)	12 (52)	0.0004
Renal crisis	6 (2)	5 (2)	1 (3)	1 (4)	0.43
Calcium channel blockers	169 (54)	99 (49)	16 (46)	13 (57)	0.28
Pulmonary hypertension specific drug therapy	0[¶,+]	0[¶,+]	17 (49)[+]	18 (78)	<0.0001
Systemic corticosteroids	41 (13)[#,¶]	60 (30)	13 (37)	7 (30)	<0.0001
Immunosuppressive therapy	34 (11)[#, ¶]	47 (23)	10 (29)	2 (9)	0.0002
NYHA functional class					<0.0001
I	236 (75)[#,¶,+]	83 (41)[¶,+]	1 (3)	2 (9)	
II	65 (21)[#,¶,+]	82 (41)[¶,+]	22 (63)	9 (39)	
III	12 (4)[#,¶,+]	33 (16)[¶,+]	12 (34)	10 (43)	
IV	0[#,¶,+]	3 (1)[¶,+]	0	2 (9)	
Estimated sPAP at T0 mmHg	28±7[¶,+]	30±7[¶,+]	53±13	57±19	<0.0001
LVEF at T0 %	64±6	65±8	64±6	61±9	0.26

Data are presented as n, mean±SD or n (%). BMI: body mass index; T0: date of inclusion, *i.e.* date of measurement of combined transfer factor of the lung for carbon monoxide and transfer factor of the lung for nitric oxide (T_{LCO}–T_{LNO}); NYHA: New York Health Association; sPAP: systolic pulmonary artery pressure; LVEF: left ventricular ejection fraction. [#]: p<0.05 *versus* Group B; [¶]: p<0.05 *versus* Group C; [+]: p<0.05 *versus* Group D.

(Group A) (table 2). In SSc patients with pulmonary hypertension (Groups C and D), V_C was significantly lower than in patients without pulmonary hypertension (Groups A and B) (table 2). SSc patients with ILD (either with or without pulmonary hypertension; Groups B and C) had lower D_{MCO} than patients without ILD (Groups A and D). It has to be noted that a total of 13 patients had a negative value of D_{MCO} (these negative values were not taken into account in the current analysis). In addition, some patients had very high D_{MCO} (a total of 11 patients had D_{MCO} 200–300% predicted and nine patients had D_{MCO} >300% predicted). These very high values were kept in the current analysis.

Regarding T_{LNO}/T_{LCO} and D_{MCO}/V_C ratios, the only significant difference found was for D_{MCO}/V_C between SSc patients without pulmonary hypertension (Groups A and B) and SSc patients with pulmonary hypertension (Group D).

There was a strong correlation between T_{LCO} and V_C (supplementary figure S1). We also found a correlation between T_{LNO} and T_{LCO}, and, although weaker, between T_{LNO} and D_{MCO}. There was a strong nonlinear relationship between the T_{LNO}/T_{LCO} and the D_{MCO}/V_C ratios. However, we were unable to find any significant correlation between D_{MCO} and V_C.

Ability of pulmonary function tests to detect pulmonary hypertension

Analysis of AUC values showed that T_{LCO} (% pred) had the highest value to detect pulmonary hypertension in our group of 572 SSc patients (figure 1 and table 3). T_{LNO} had a slightly, but nonsignificantly lower AUC value. By contrast, V_C and D_{MCO} had a significantly lower diagnostic value than both T_{LCO} and T_{LNO} (figure 1 and table 3). The T_{LNO}/T_{LCO} and the D_{MCO}/V_C ratios had the worst diagnostic values for pulmonary hypertension (figure 1 and table 3).

TABLE 2 Pulmonary function tests of the studied population at inclusion, divided into four groups: systemic sclerosis (SSc) patients without any pulmonary involvement, *i.e.* neither interstitial lung disease (ILD) nor pulmonary hypertension (Group A), SSc patients with ILD but without pulmonary hypertension (Group B), SSc patients with both ILD and pulmonary hypertension (Group C), and SSc patients without ILD but with pulmonary hypertension (Group D)

	Group A	Group B	Group C	Group D	p-value
Subjects	313	201	35	23	
FEV_1					
L	2.49±0.66$^{\#,\P,+}$	2.28±0.63^{+}	2.00±0.64	1.87±0.63	<0.0001
% pred	102±20$^{\#,\P,+}$	91±20	83±20	86±22	<0.0001
FVC					
L	3.21±0.79$^{\#,\P,+}$	2.88±0.85	2.64±0.87	2.56±0.89	<0.0001
% pred	111±20$^{\#,\P,+}$	95±22	90±22	97±23	<0.0001
FEV_1/FVC % (actual)	78±8$^{\#,+}$	80±8	77±12	73±7	<0.0001
SVC					
L	3.32±0.80$^{\#,\P,+}$	2.93±0.87	2.65±0.77	2.52±0.68	<0.0001
% pred	114±20$^{\#,\P,+}$	96±23	91±21	99±22	<0.0001
TLC					
L	5.20±0.95$^{\#,\P}$	4.76±1.31	4.63±1.13	4.87±1.01	<0.0001
% pred	105±15$^{\#,\P}$	89±19	86±17	97±18	<0.0001
FRC					
L	3.02±0.63$^{\#}$	2.79±0.86	2.87±0.83	2.83±0.73	0.023
% pred	109±21$^{\#,\P}$	96±24	94±22	101±22	<0.0001
RV					
L	1.97±0.52$^{\#}$	1.80±0.64^{+}	1.84±0.52	2.19±0.80	0.001
% pred	106±25$^{\#,\P}$	91±28	90±24	107±38	<0.0001
T_{LCO}					
mL·mmHg^{-1}·min^{-1}	18.4±5.6$^{\#,\P,+}$	14.6±6.0$^{\P,+}$	9.6±3.3	9.7±4.3	<0.0001
% pred	81±22$^{\#,\P,+}$	63±22$^{\P,+}$	44±19	49±21	<0.0001
T_{LNO}					
mL·mmHg^{-1}·min^{-1}	91.0±27.7$^{\#,\P,+}$	71.9±26.5$^{\P,+}$	51.2±18.0	48.9±17.0	<0.0001
% pred	67±22$^{\#,\P,+}$	56±23$^{\P,+}$	43±17	40±14	<0.0001
T_{LNO}/T_{LCO}	5.06±1.20	5.16±1.28	5.46±1.42	5.78±2.61	0.036
Hb g·100 mL^{-1}	13.4±1.4^{+}	13.1±1.4	13.0±2.0	12.5±1.7	0.014
V_C					
mL	45±18$^{\#,\P,+}$	37±18¶	25±11	27±15	<0.0001
% pred	72±25$^{\#,\P,+}$	58±26¶	43±23	47±24	<0.0001
D_{MCO}					
mL·mmHg^{-1}·min^{-1}	104±105$^{\#}$	74±38	85±97	105±244	0.008
% pred	106±101$^{\#}$	73±38	73±60	138±345	0.0008
D_{MCO}/V_C	2.77±5.19^{+}	2.38±1.87^{+}	4.90±8.29	7.66±24.5	0.002

Data are presented as n or mean±SD. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; SVC: slow vital capacity; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; T_{LCO}: transfer factor of the lung for carbon monoxide; T_{LNO}: transfer factor of the lung for nitric oxide; Hb: haemoglobin concentration; V_C: alveolar capillary blood volume; D_{MCO}: membrane conductance (diffusing capacity) for carbon monoxide. $^{\#}$: p<0.05 *versus* Group B; ¶: p<0.05 *versus* Group C; $^{+}$: p<0.05 *versus* Group D.

The sensitivities and specificities of all parameters for the detection of pulmonary hypertension are given in table 3, as well as the positive and negative predictive values for different thresholds. For the optimal thresholds determined with the Youden methods, the negative predictive values of T_{LCO} (% pred) and T_{LNO} (% pred) were very high, indicating that a T_{LCO} and/or a T_{LNO} above these thresholds virtually excludes pulmonary hypertension. Nevertheless, the low positive predictive values indicate that "abnormal" T_{LCO} or T_{LNO} were often present in the absence of pulmonary hypertension.

Follow-up

The median follow-up time for the entire SSc cohort (n=572) was 24 months. The overall survival of the cohort is shown in figure 2. Patients with pulmonary hypertension (Groups C and D) had a significantly poorer survival than those without pulmonary hypertension (Groups A and B) (figure 2). Of note, also in SSc patients without pulmonary hypertension, the presence of ILD did not affect survival (figure 2).

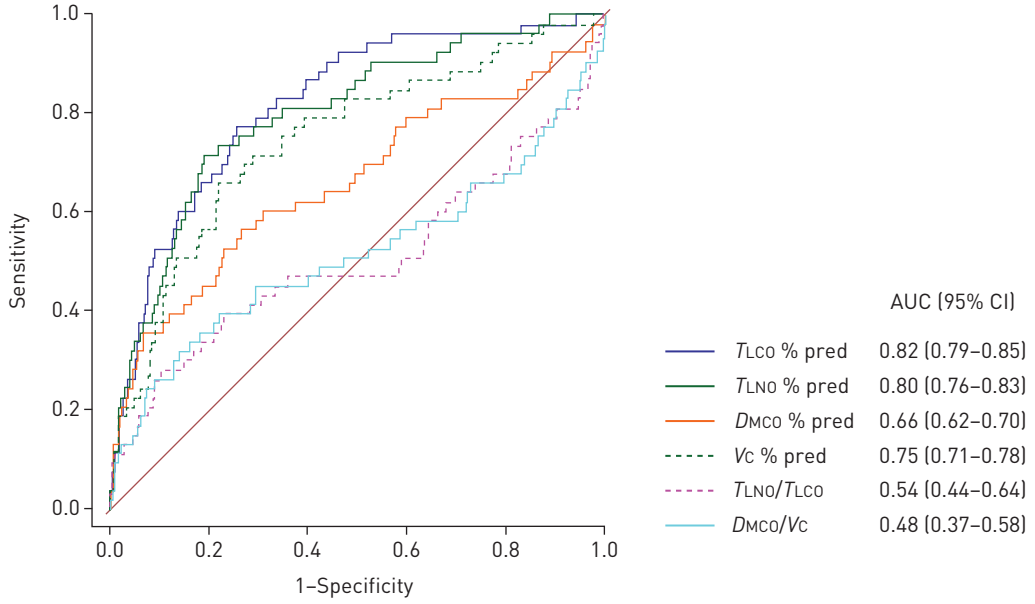


FIGURE 1 Receiver operating characteristic (ROC) curves of transfer factor of the lung for carbon monoxide (*T*LCO, % pred), transfer factor of the lung for nitric oxide (*T*LNO), membrane conductance (diffusing capacity) for carbon monoxide (*D*MCO), alveolar capillary blood volume (*V*C), *T*LNO/*T*LCO ratio and *D*MCO/*V*C ratio for discrimination of pulmonary hypertension (n=58) in comparison with the absence of pulmonary hypertension (n=514) in patients with systemic sclerosis. Area under the ROC curve (AUC) values are also given.

Univariate and multivariate analyses of factors associated with survival are presented in table 4. In univariate analysis, *T*LCO, *T*LNO, *V*C and *D*MCO were all associated with survival. In multivariate analysis, only *T*LCO, forced vital capacity, age at onset and pulmonary hypertension were associated with survival, with *T*LCO having the highest hazard ratio.

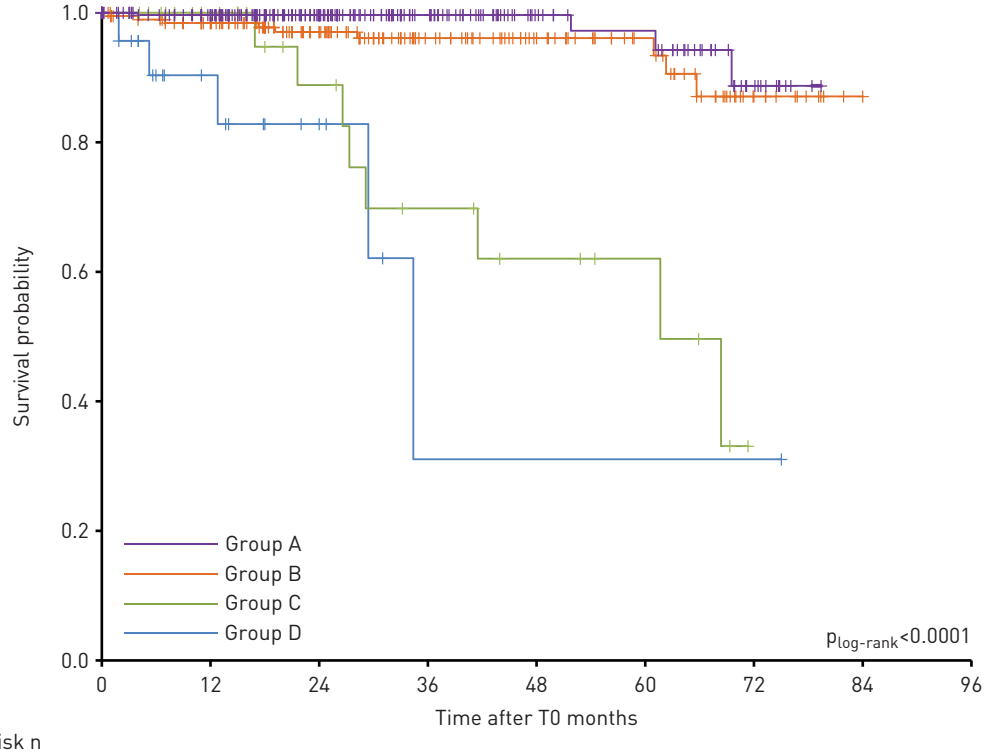
Discussion

The relevance of *T*LNO measurement and *T*LCO partitioning into *D*MCO and *V*C for the identification of pulmonary hypertension in SSc is debated, with sparse and conflicting results based on small, single-centre

TABLE 3 Performance of the tested variables with different thresholds for the probability of pulmonary hypertension

	AUC (95% CI)	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %
TLCO % pred	0.82 (0.79–0.85)	60%#	77	74	24	97
		LLN2.5%	72	66	19	96
		LLN5%	78	58	17	96
TLNO % pred	0.80 (0.76–0.83)	45%#	72	81	28	96
		LLN2.5%	90	30	12	96
		LLN5%	93	24	12	97
VC % pred	0.75 (0.71–0.78)	50%#	66	77	23	96
		LLN2.5%	74	59	17	95
		LLN5%	79	46	14	95
DMCO % pred	0.66 (0.62–0.70)	61%#	57	74	19	94
		LLN2.5%	40	83	20	93
		LLN5%	45	77	17	93
TLNO/TLCO	0.54 (0.44–0.64)	4#	19	94	26	92
DMCO/VC	0.48 (0.37–0.58)	1#	24	89	20	91

AUC: area under the receiver operating characteristic (ROC) curve; NPV: negative predictive value; PPV: positive predictive value; *T*LCO: transfer factor of the lung for carbon monoxide; LLN: lower limit of normal; LLN2.5%: results from 2.5% of healthy individuals will be below this cut-off; LLN5%: results from 5% of healthy individuals will be below this cut-off; *T*LNO: transfer factor of the lung for nitric oxide; *V*C: alveolar capillary blood volume; *D*MCO: membrane conductance (diffusing capacity) for carbon monoxide. #: optimal threshold of pulmonary hypertension probability assessed according to the Youden index of ROC curve analysis.



At risk n								
Group A	313	249	145	80	46	33	11	0
Group B	201	169	118	73	52	36	12	1
Group C	35	24	15	10	7	5	0	0
Group D	23	12	6	1	1	1	1	0

FIGURE 2 Kaplan–Meier estimates of overall survival in patients with systemic sclerosis (SSc) but with no pulmonary involvement, *i.e.* neither interstitial lung disease (ILD) nor pulmonary hypertension (Group A), SSc patients with ILD but without pulmonary hypertension (Group B), SSc patients with both ILD and pulmonary hypertension (Group D), and SSc patients without ILD but with pulmonary hypertension (Group D). T0: date of inclusion, *i.e.* date of measurement of combined transfer factor of the lung for carbon monoxide and transfer factor of the lung for nitric oxide (T_{LCO}–T_{LNO}).

series of patients [18–20]. Here, we analysed a large, multicentre series of unselected patients with well-defined SSc. Although we used recently refined equations for the calculation of V_C and D_{MCO}, we found that T_{LNO} was equivalent to T_{LCO}, while D_{MCO} and V_C had a lower capability than T_{LCO} to detect pulmonary hypertension. In addition, we found that in SSc patients, T_{LCO} was a stronger predictor of overall mortality than T_{LNO}, V_C or D_{MCO}.

T_{LCO} has emerged as one of the most sensitive and specific routine pulmonary function tests that have been evaluated for identifying pulmonary hypertension in patients with SSc [10, 27, 28]. This has been demonstrated in selected SSc patients: in SSc patients with T_{LCO} <60% predicted and disease duration >3 years, T_{LCO} contributes to predicting pulmonary hypertension through the DETECT algorithm [29]. This has also been shown in unselected SSc patients, in whom T_{LCO} contributes to predicting mean pulmonary artery pressure [30]. The analysis of our large series of unselected SSc patients confirms that T_{LCO} is associated with the presence of pulmonary hypertension. A recent review recalled that an abnormally low T_{LNO}, defined by a value lower than the lower limit of normal (below the 2.5th percentile ($LLN_{2.5\%}$)), is more sensitive than an abnormally low T_{LCO} to identify the presence of "cardiopulmonary diseases" [31]. We also found that a T_{LNO} <$LLN_{2.5\%}$ was more sensitive than a T_{LCO} <$LLN_{2.5\%}$ for the identification of pulmonary hypertension (table 3), but at the price of much lower specificity for T_{LNO} than for T_{LCO}.

Our study confirms that although the negative predictive value of T_{LCO} for the presence of pulmonary hypertension is very high, the positive predictive value is poor, reflecting the fact that many patients with "low" T_{LCO} do not have pulmonary hypertension. This is largely related to the presence of ILD, a frequent complication of SSc, which is also associated with a low T_{LCO} [12, 32].

Although the interpretation of V_C and D_{MCO} calculated with the Roughton–Forster model remains controversial [33], these physiological parameters are supposed to discriminate between vascular and

TABLE 4 Univariate and multivariate analyses of selected biological, clinical and functional factors associated with survival

	Univariate		Multivariate	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Limited cutaneous subtype	1.45 (0.58–3.61)	0.43		
Age at onset >60 years	2.47 (1.10–5.53)	0.03	2.79 (1.23–6.35)	0.01
Haemoglobin <12 g·dL^{-1}	2.24 (0.97–5.16)	0.06		
Pulmonary hypertension	11.40 (5.27–24.66)	<0.0001	4.71 (2.12–10.48)	0.0001
Interstitial lung disease	2.00 (0.89–4.49)	0.10		
LVEF <55%	1.49 (0.35–6.41)	0.59		
FVC <LLN$_{5\%}$	4.49 (2.06–9.79)	0.0002	3.02 (1.33–6.84)	0.008
T_{LCO} <60% pred	23.21 (5.48–98.39)	<0.0001	10.01 (2.16–46.48)	0.003
T_{LNO} <45% pred	6.46 (2.91–14.32)	<0.0001		
V_C <50% pred	7.69 (2.86–20.63)	<0.0001		
D_{MCO} <61% pred	4.93 (2.07–11.73)	0.0008		

LVEF: left ventricular ejection fraction; FVC: forced vital capacity; LLN: lower limit of normal; LLN$_{5\%}$: results from 5% of healthy individuals will be below this cut-off; T_{LCO}: transfer factor of the lung for carbon monoxide; T_{LNO}: transfer factor of the lung for nitric oxide; V_C: alveolar capillary blood volume; D_{MCO}: membrane conductance (diffusing capacity) for carbon monoxide.

alveolo-capillary membrane alterations. It was recently reported that V_C is decreased in patients with pulmonary hypertension without SSc [34]. In accordance with this finding, we report that V_C was lowest when pulmonary hypertension was present in SSc patients. Nevertheless, in our group of SSc patients, we also found that V_C was lower in patients with ILD without pulmonary hypertension than in patients without any pulmonary involvement. Although SSc patients with ILD had preserved lung volumes and normal estimated systolic pulmonary artery pressure, our results suggest that they may have either pulmonary vascular destruction and/or pulmonary vasculopathy.

Several studies have reported a reduction in D_{MCO} in pulmonary vascular diseases, either associated [18] or not [35] with SSc. Although thickening of the alveolo-capillary membrane due to remodelling of the pulmonary vasculature cannot be excluded, this has been previously interpreted as a loss of functional gas exchange area that accompanies the loss of V_C, a hypothesis supported by a strong correlation between V_C and D_{MCO} [34]. Here, we used very recently refined equations, and we did not find any significant correlation between V_C and D_{MCO} [21]. In addition, we found that D_{MCO} was significantly lower in patients with ILD (independently of the presence of pulmonary hypertension) than in patients without. This therefore reinforces the view that D_{MCO} provides information regarding a "thickening" of the alveolo-capillary membrane or a decrease in lung area.

An increase in T_{LNO}/T_{LCO} and D_{MCO}/V_C ratios has been found in patients with pulmonary microvascular disease [36]. Although there was a tendency towards such a finding in our series of SSc patients, we found only slight differences in terms of T_{LNO}/T_{LCO} and D_{MCO}/V_C ratios between patients with pulmonary hypertension and those without. Regarding the D_{MCO}/V_C ratio, the absence of a significant difference between groups is likely due to the very high standard deviation, as D_{MCO} was very high in some patients.

In agreement with previous reports, we also found here that survival of SSc patients with ILD but without pulmonary hypertension was similar to that of patients without any pulmonary involvement [6, 37]. Nevertheless, SSc patients with pulmonary hypertension either with or without ILD had a similar poor survival. In other words, survival was linked to pulmonary hypertension rather to ILD in SSc. As prompt therapy of pulmonary hypertension in SSc has been shown to be beneficial in patients without as well as with ILD [7, 8], this reinforces the pertinence of finding a test that can identify pulmonary hypertension independently of the presence of ILD in SSc patients. Our results demonstrate that T_{LCO} remains better than V_C and D_{MCO} calculated with the modified one-step Roughton–Forster technique for that purpose.

Strengths of this study include the real-life nature of the patient series and the multicentre assessment. Our cohort represents the largest series of patients with SSc where diffusion impairments have been assessed by combined T_{LCO} and T_{LNO} measurements. The group of unselected SSc patients analysed here is likely to be representative of SSc patients followed in most referral centres because in the seven participating centres, each patient with SSc has an annually scheduled follow-up that comprises echocardiography and pulmonary function tests. 41% of our patients had some degree of ILD; this percentage is similar to that reported in previous SSc cohorts [38] and ILD was more than twice as

common among patients with diffuse cutaneous subtype than among those with limited subtype. The prevalence of pulmonary hypertension (10.1%) found here is also close to that reported in previous series [3, 11, 39, 40].

Limitations of this study include the retrospective analysis and the observational nature of our cohort. We were therefore unable to identify in each of the seven participating centres the number of SSc patients who were not included in the current study because of missing data on echocardiography and/or right heart catheterisation and/or HRCT and/or pulmonary function tests. There are also limitations in terms of the assessments that were directed by clinical practice and did not follow a predefined study protocol. Some important complications were not ascertained (*e.g.* gastrointestinal tract involvement) and some others were impossible to collect in some centres (*e.g.* Rodnan score, which assesses skin thickness in the diffuse cutaneous subtype of SSc). We were unable to collect haemodynamic data for all patients who underwent right heart catheterisation; these data were therefore omitted and only the item "pre-capillary pulmonary hypertension according to right heart catheterisation" was collected in all patient files. Nevertheless, echocardiographic estimation of systolic pulmonary artery pressure was systematically reviewed and collected.

In conclusion, our study provides evidence that the lower T_{LCO} in SSc patients with pulmonary hypertension, compared with SSc patients without pulmonary hypertension, is attributable to the lower V_C in those with pulmonary hypertension. Nevertheless, we failed to demonstrate any clinical usefulness of the T_{LCO} partition into D_{MCO} and V_C for the identification of pulmonary hypertension in patients with SSc. Our results are at variance with some results based on small and/or selected populations, thus highlighting the importance of cohort representativeness for the applicability of study results to a general population of SSc patients.

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