Diffusing Pulmonary Capacity Measured During Effort: A Possible Early Marker of Pulmonary Involvement In Systemic Sclerosis

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ABSTRACT: **Background:** Interstitial lung involvement is common and potentially limits the quality of life in patients with systemic limited sclerosis (SScl).

Objectives: To study the lung carbon monoxide diffusion (DLCO) measured during effort in order to identify a possible subclinical impairment.

Methods: We enrolled 20 SScl patients without interstitial lung involement and 20 healthy controls. At enrolment all subjetcs underwent plethysmography, DLCO by single-breath technique, and evaluation of pulmonary blood flow (Qc) with the rebreathing CO_2 method. Skin involvement in the SScl patients was rated using the modified Rodman skin score (mRSS). During exercise on a cycle ergometer, DLCO, DLCO/alveolar volume (Kco) and Qc were calculated at 25% and 50% of predicted maximum workload (25% pmw and 50% pmw).

Results: At baseline two groups did not differ in age, body mass index, lung function or Qc. In the controls, DLCO, Kco and DLCO/Qc measured at 25% pmw and 50% pmw were significantly higher than in SScl patients, while Qc was not different. Based on response to effort, SScl patients were divided into two groups: responders, with an increase of $DLCO_{25\%pmw}$ and $DLCO_{50\%pmw}$ at least 5% and 10% respectively, and non-responders. The non-responders showed greater skin involvement and significantly reduced DLCO, Kco and DLCO/Qc values at rest than responders.

Conclusions: Moderate effort in SScl patients may reveal a latent impairment in gas diffusion through the alveolar/capillary membrane, thus confirmig that exertional DLCO can identify lung damage at an earlier stage than DLCO at rest.

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KEY WORDS: systemic sclerosis (SSc), interstitial lung disease (ILD), 6 minute walk test, carbon monoxide diffusion capacity of lung (DLCO)

Systemic sclerosis (SSc) is unique in that it is characterized by the features of three distinct pathophysiological processes: cellular and humoral autoimmunity, vascular injury, and tissue fibrosis. Functional and structural vascular injury is often the earliest sign and may occur years before the other manifestations. Moreover, fibrosis can occur as a result of the excessive accumulation of collagen and extracellular matrix components. Genetic factors may play a role in the pathogenesis of the disease by affecting host susceptibility or modifying its clinical presentation and organ damage [1].

Impaired lung carbon monoxide diffusing capacity (DLCO) has been reported frequently [2,3] and can be affected by two distinct mechanisms. One is pulmonary fibrosis, which decreases alveolar volume (VA), directly affects the rate of CO uptake and is particularly common in patients with diffuse SSc in whom DLCO impairment is often due to associated interstitial lung disease (ILD) [4]; the other is pulmonary capillary dysfunction, where pulmonary artery hypertension (PAH) represents the ultimate stage of microvascular impairment. Since a reduction in DLCO (in the absence of ILD and PAH) has been reported, especially in patients with limited forms of SSc (SScl) [5], DLCO reflects the underlying pulmonary vasculopathy [6] and is a well-known major predictive factor and early marker of PAH [2,3,5,6]. The symptoms in SSc patients often include dyspnea upon exertion, fatigue and reduced exercise tolerance but are only recognized when pulmonary involvement has produced irreversible damage.

Interstitial lung involvement is frequent and potentially limits the quality of life of SSc patients. Our belief that its early diagnosis would allow early treatment and limit the consequences led us to search for a parameter of early alveolar/capillary damage that could be used as a test in routine clinical practice. The aim of this study was to investigate the behavior of the respiratory system under stress based on the DLCO measured during effort in order to identify a possible subclinical impairment.

PATIENTS AND METHODS

Forty subjects were enrolled at L. Sacco Hospital, Milan, Italy, between 14 November 2013 and 30 May 2014: 20 SScl patients referred by a rheumatologist to the Respiratory Function

Laboratory and 20 healthy controls matched in living habits, age and body mass index (BMI). The study was approved by the local Human Ethics Committee and conducted in accordance with the ethical guidelines of the 1975 Helsinki Declaration, and all participants gave their informed consent following an explanation on the study procedures and their risks.

INCLUSION AND EXCLUSION CRITERIA

Patients were included in the study if they had a diagnosis of SScl, normal respiratory function, normal arterial blood pressure, normal pulmonary high resolution computed tomography (HRCT) and normal electrocardiographic findings. Exclusion criteria were smoking, lung disease, heart disease, obesity (BMI of ≥ 30), anemia, diabetes mellitus, or the presence of musculoskeletal diseases that could limit exercise.

MODIFIED RODNAN SKIN SCORE (MRSS)

Skin involvement in the patients with SScl was rated using the mRSS before their respiratory function was tested. The body surface was arbitrarily divided into 17 areas: face, anterior chest, abdomen, fingers (right and left separately), forearms, upper arms, thighs, lower legs and dorsum of hands and feet – each scored from 0 to 3 according to the degree of skin involvement as assessed by manual palpation [7]. The extent of involvement correlates with that of the internal organs [8].

PULMONARY FUNCTION TESTS

Lung volume and dynamic spirometric parameters were assessed by plethysmography (VMAX227 Autobox V6200™, Sensor Medics, Yorba Linda, CA, USA) in accordance with the criteria of the European Respiratory Society (ERS) [9]. DLCO was measured using the single-breath technique and a mixture of carbon monoxide and methane, as recommended by the ERS [10], and was adjusted for the level of carboxyhemoglobin (COHb) measured with a blood gas analyzer (Critical Care Laboratory Synthesis 35™, Instrumentation Laboratory, Paderno Dugnano, Italy) using the following equation [11]:

Adjusted DLCO = COHb - measured DLCO
(1+[%COHb/100])

The carbon monoxide transfer factor coefficient (Kco) was derived from the following equation:

Kco = DLCO/alveolar volume.

EXERCISE TEST

The exercise tests were performed in accordance with a standardized cardiopulmonary exercise test procedure [12]. After the oxygen and carbon dioxide sensors had been calibrated, the subjects were asked to sit on an electromagnetically braked cycle ergometer (Ergometric 800™, Sensor Medics) with the saddle adjusted to avoid maximal knee extension. After 3 minutes sitting on the ergometer, subjects began the exercise with a 3 minute warm-up at 0 watts, followed by a progressively increasing ramp protocol of 10–25 watts/min depending on the subject's anthropometric data so as to ensure an exercise time of 8–12 minutes. All subjects had to maintain a cycling frequency of 60 rpm as indicated by a digital display on the ergometer monitor.

DLCO and effective pulmonary blood flow (Qc) were calculated using the rebreathing CO_2 method (R-CO_2) at rest and at 25% and 50% of predicted maximum workload (25% pmw, 50% pmw) [13,14]. R-CO_2 was evaluated using Fick's formula: Qc = $VCO_2/CvCO_2$ - $CaCO_2$, where VCO_2 is CO_2 production, $CvCO_2$ is CO_2 content in mixed venous blood, and $CaCO_2$ is arterial CO_2. VCO_2 was obtained by a computer calculation (Vmax 229, Sensor Medics), $CvCO_2$ was obtained after 10 seconds of respiration in a mixture of 7% CO_2 in O_2, and $CaCO_2$ was obtained from the CO_2 end-tidal capnogram.

The subjects were continuously monitored by a 12-lead electrocardiogram (ECG) and pulse oximeter (Pulse Oximeter 8600™, Nonin Medical Inc, Plymouth, Mn, USA). Blood pressure was measured at 2 minute intervals.

The end test criteria were symptoms such as unsustainable dyspnea or leg fatigue, chest pain, ECG ST-segment depression, systolic blood pressure > 220 mmHg or SaO_2% < 88%.

STATISTICAL ANALYSIS

The data of the SScl patients and healthy controls were compared using an unpaired *t*-test. One-way analysis of variance, the Mann-Whitney U-test and Spearman's rank correlations were used when appropriate. All variables entered in the analyses were expressed as mean values ± SD; a P value < 0.05 was considered statistically significant. The data were stored and analyzed using the SPSS v. 6.1 software (SPSS, Chicago, IL).

RESULTS

The duration of SScl in the patients was 4 ± 6 years at the time of enrolment; 19/20 SScl patients had antinuclear antibodies (ANA), 15/20 anticentromere antibodies, and 16/20 had abnormal nail-fold capillary microscopy findings. There were no differences between the patients and controls in terms of age (50 ± 5 vs. 51 ± 4 years), BMI (25 ± 3 vs.24 ± 4 kg/m^2), socioeconomic status, or lung function and Qc at rest [Table 1].

None of the enrolled subjects had any difficulty completing the tests. Figure 1 shows the DLCO, Kco, DLCO/Qc and Qc values in the two groups before exercise, and at 25% and 50% of predicted maximum workload. DLCO, Kco and DLCO/Qc at the three time points were significantly higher in the controls than in the SScl patients ($DLCO_{25\%pmw}$ 115 ± 13% vs. 94 ± 15%, P < 0.0001; $DLCO_{50\%pmw}$ 133 ± 15% vs.100 ± 13%, P < 0.0001; $Kco_{25\%pmw}$ 118 ± 6% vs. 95 ± 12%, P < 0.0001; $Kco_{50\%pmw}$ 136 ± 15% vs. 101 ± 13%, P < 0.0001; $DLCO/Qc_{25\%pmw}$ 7.5 ± 0.4 vs. 5.0 ± 0.6 ml/L^{-1} mmHg^{-1}, P < 0.0001;

Table 1. Baseline demographics, lung function data and cardiac output of patients with systemic sclerosis (SSc) and healthy controls [20]

	SSc (n=20)	Healthy (n=20)	*P* value
Age (years)	50 ± 5	51 ± 4	NS
Gender (M/F)	2/18	3/17	NS
Predicted VC (%)	89 ± 3	91 ± 6	NS
FEV1 (%)	87 ± 4	89 ± 5	NS
TLC (%)	88 ± 5	91 ± 6	NS
RV (%)	91 ± 6	88 ± 4	NS
DLCO (%)	90 ± 13	93 ± 7	NS
Kco (%)	92 ± 12	93 ± 4	NS
Qc (L/min)	4.7 ± 0.5	4.9 ± 0.3	NS
DLCO/Qc (ml/[L x mmHg])	4.8 ± 0.7	5.1 ± 0.4	NS

Mean values ± SD, $P < 0.05$

VC = vital capacity, FEV1 = forced expiratory volume in 1 second, TLC = total lung capacity, RV = residual volume, DLCO = diffusion lung capacity for carbon monoxide, Kco = carbon monoxide transfer factor coefficient, Qc = effective pulmonary blood flow, NS = not significant

Table 2. Lung function and cardiac output in 20 patients with systemic sclerosis (SSc) and 20 healthy controls before exercise and at 25% and 50% of theoretical maximum workload

	Rest	25%	50%	*P*
SSc patients				
Predicted DLCO (%)	90 ± 13	94 ± 15	100 ± 13*	NS
Kco (%)	92 ± 12	95 ± 12	101 ± 13*	NS
DLCO/Qc (ml/[L x mmHg])	4.8 ± 0.7	5 ± 0.6	4.9 ± 0.5	NS
Qc (L/min)	4.7 ± 0.5	7.3 ± 0.4*	10.8 ± 0.5§	0.0001
Healthy controls				
Predicted DLCO (%)	93 ± 7	115 ± 13‡	133 ± 15‡	0.0001
Kco (%)	93 ± 4	118 ± 6‡	136 ± 15‡	0.0001
DLCO/Qc (ml/[L x mmHg])	5.1 ± 0.4	7.5 ± 0.4‡	10.5 ± 0.6‡	0.0001
Qc (L/min)	4.9 ± 0.3	7.5 ± 0.6‡	11 ± 0.6‡	0.0001

Mean values ± SD, $P < 0.05$

*0.05

§0.001

‡0.0001

DLCO = diffusion lung capacity for carbon monoxide, Kco = carbon monoxide transfer factor coefficient, Qc = effective pulmonary blood flow, NS = not significant

DLCO/Qc$_{50\%pmw}$ 10.5 ± 0.6 vs. 4.9 ± 0.5 ml/L^{-1} mmHg^{-1}, $P < 0.001$), but there were no statistically significant differences in Qc (Qc$_{25\%pmw}$ 7.5 ± 0.6 vs. 7.3 ± 0.4 L/min, not significant; Qc$_{50\%pmw}$ 11 ± 0.6 vs. 10.8 ± 0.5 L/min, NS).

In the SScl patients, there was only a small increase in DLCO and Kco at 50% of predicted maximum workload (respectively 90 ± 13% vs. 100 ± 13%, $P < 0.05$, and 92 ± 12% vs. 101 ± 13%, $P < 0.05$), and no statistically significant changes in DLCO/Qc throughout the test [Table 2].

According to their responses to effort, the SScl patients were divided into two groups: 9 responders in whom DLCO$_{25\%pmw}$ and DLCO$_{50\%pmw}$ increased by at least 5% and 10% respectively, and 11 non-responders in whom the increases were less than 5% and 10% respectively.

At rest, the non-responders showed significantly reduced DLCO (86 ± 9% vs. 94 ± 8%, $P < 0.05$), Kco (87 ± 7% vs. 94 ± 8%, $P < 0.05$) and DLCO/Qc (4.3 ± 0.4 vs. 4.9 ± 0.5 ml/L mmHg, $P < 0.001$) and greater skin involvement (mRSS 6.1 ± 5.6 vs. 1.3 ± 1, $P < 0.0001$) [Table 3]. The percentage increase from baseline in DLCO, Kco and DLCO/Qc at 25% and 50% of predicted maximum workload negatively correlated with mRSS (r -0.60, $P < 0.01$, r = -0.5, $P < 0.05$, and r = -0.65, $P < 0.005$). There were no significant correlations between DLCO, Kco and DLCO/Qc measured at 25% and 50% of predicted maximum workload and age, duration of disease or the presence of autoantibodies.

DISCUSSION

DLCO in healthy subjects increases during exercise as a function of the expansion of the pulmonary capillary bed due to the increase in Qc, the exchange surface, and an increase in lung volume that occurs with adaptation to physical stress [15]. Manier et al. [16] showed that the increase in DLCO reaches a plateau corresponding to the maximum diffusion capacity; this is probably due to Qc limitation and not to the inability of

Figure 1. Values in patients with systemic sclerosis (SSc) and healthy controls before exercise and at 25% and 50% of predicted maximum workload. **[A]** DLCO, **[B]** Kco (*$P < 0.0001$), **[C]** TLCO/Qc (*$P < 0.0001$), **[D]** Qc

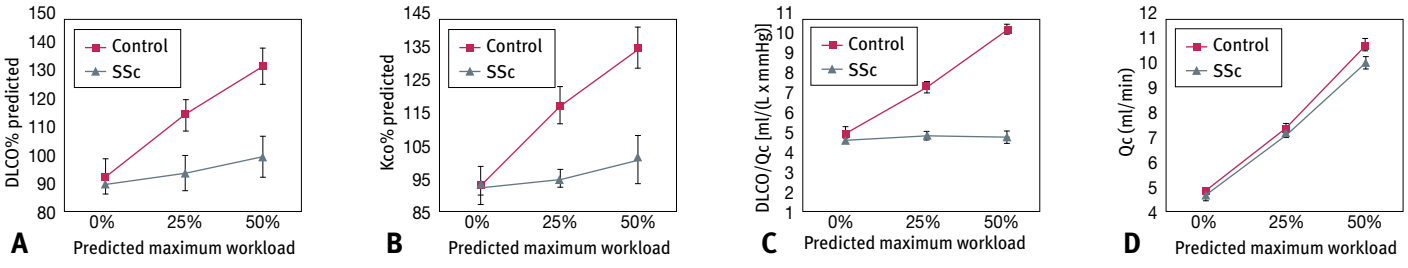

Table 3. Lung function, cardiac output and modified Rodnan Skin Score in responders and non-responders

	SSc patients		
	Non-responders (n=11)	Responders (n=9)	*P*
Predicted DLCO (%)	86 ± 9	94 ± 8	0.05
Kco (%)	87 ± 7	94 ± 8	0.05
DLCO/Qc (ml/[L x mmHg])	4.3 ± 0.4	4.9 ± 0.5	0.001
Qc (L/min)	4.7 ± 0.6	4.8 ± 0.5	NS
RV (%)	91 ± 4	91 ± 6	NS
TLC (%)	89 ± 6	88 ± 4	NS
VC (%)	89 ± 4	88 ± 5	NS
FEV1 (%)	88 ± 6	89 ± 5	NS
MRSS	6.1 ± 5.6	1.3 ± 1	0.0001

Mean values ± SD, $P < 0.05$

VC = vital capacity, FEV1 = forced expiratory volume in 1 second, TLC = total lung capacity, RV = residual volume, DLCO = diffusion lung capacity for carbon monoxide, Kco = carbon monoxide transfer factor coefficient, Qc = effective pulmonary blood flow, mRSS = modified Rodnan Skin Score, NS = not significant

the pulmonary membrane to ensure adequate gas exchange. The increase in DLCO from rest to maximum working load in healthy subjects can be more than twice the increase in Qc due to the recruitment of poorly perfused capillaries and alveolar distension. The increase in capillary volume during lung expansion is parabolic: it peaks at 60% of alveolar volume, but decreases between 60% and 100% probably because the stretched lung tissue compresses pulmonary capillaries. The effectiveness of the recruitment is indicated by the variation in DLCO/Qc when exercise load increases [17]. The relationship between DLCO and Qc is commonly used as an index of alveolar gas exchange efficiency [18,19].

Serially measured decreases in forced vital capacity and DLCO have a negative impact on prognosis [20] and even small changes within the first 6–12 months of observation may translate into major survival differences in the subsequent years.

The impaired DLCO in SSc patients [21,22] may be caused by two distinct mechanisms: pulmonary capillary dysfunction or pulmonary fibrosis. The latter is particularly common in patients with diffuse SSc in whom DLCO impairment is often due to ILD; thus DLCO may reflect an underlying pulmonary vasculopathy [23]. DLCO impairment has also been linked to altered nail-fold capillary microscopy findings in SSc patients with Raynaud's phenomenon [20].

Trad and co-authors [24] retrospectively studied 145 patients with limited skin sclerosis and a high prevalence of low DLCO values, which they attributed to greater involvement of the alveolar/capillary membrane, and concluded that isolated DLCO impairment is significantly more frequent in patients with SScl.

In our study all DLCO values were adjusted for carboxyhemoglobin to minimize the influence of CO back pressure and differences in hemoglobin concentrations. Although we used Fick's indirect method, the CO_2 rebreathing method used to study Qc correlates well with the direct method in healthy subjects and those with limited alterations in pulmonary ventilation.

Our data revealed no significant differences in plethysmography parameters, Qc, or diffusion capacity measured at rest between SScl patients and healthy subjects. During effort, DLCO increased very little in the SScl group (by 4% and 10% at respectively 25% and 50% of predicted maximum workload), unlike in healthy subjects (18% and 25%). The reduced increase of DLCO and Kco during exercise (at 25 and 50% of predicted maximum workload) in SScl patients may be due to a reduction in the diffusion capacity of the alveolar/capillary membrane, as confirmed by the lack of increase in DLCO/Qc during exercise.

The greater skin involvement in SScl patients and the lower increase in DLCO, Kco and DLCO/Qc during exercise, and the negative correlation between mRSS and the increase in DLCO, Kco and DLCO/Qc during exercise, provide further evidence that more extensive skin involvement is indicative of greater visceral involvement [8].

LIMITATIONS

The method described here can only be used in relatively young, cooperative and well-coordinated patients, especially when measuring DLCO, because the single-breath method used by us requires maintaining 10 seconds of apnea during exertion. Moreover, the sample of patients examined in our study is small, and a larger number of patients is needed to obtain more significant data.

CONCLUSIONS

Our data suggest that moderate effort in SScl patients may reveal a latent impairment in gas diffusion through the alveolar/capillary membrane, thus confirming that exertional DLCO can identify lung damage at an earlier stage than DLCO at rest. DLCO measurements during exertion could be used for the early diagnosis of pulmonary involvement in SScl patients. Furthermore, since more extensive skin involvement seems to be associated with an earlier pulmonary involvement this could indicate which patients need to be monitored frequently.

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