Determinants of impairment in lung diffusing capacity in patients with systemic sclerosis

G. Guarnieri¹, E. Zanatta², P. Mason¹, M.C. Scarpa¹, E. Pigatto², P. Maestrelli¹, F. Cozzi²

¹Department of Cardiologic, Thoracic and Vascular Sciences, and ²Rheumatology Unit, Department of Medicine, University of Padova, Padova, Italy.

Gabriella Guarnieri, MD
Elisabetta Zanatta, MD
Paola Mason, MD
Maria Cristina Scarpa, PhD
Erika Pigatto, MD
Piero Maestrelli, MD
Franco Cozzi, MD

Please address correspondence to:
Prof. Piero Maestrelli,
Dipartimento di Scienze Cardiologiche, Toraciche e Vascolari,
Università degli Studi di Padova,
via Giustiniani 2,
35128 Padova, Italy.
E-mail: piero.maestrelli@unipd.it

Funding: this work was supported by grants from the University of Padova (60A07-5575/13; CPDA105994/10) and by the Associazione Ricerca Cura Asma (ARCA), Padova, Italy.

Competing interests: none declared.

ABSTRACT

Objective. Lung diffusing capacity for carbon monoxide (DLCO) is impaired in interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH) associated to systemic sclerosis (SSc), but the mechanism of DLCO reduction remains controversial. We hypothesised that the determinants of DLCO impairment differ in interstitial or vascular involvement of the lung of SSc patients.

Methods. DLCO was partitioned into alveolar-capillary membrane conductance (Dm) and pulmonary capillary blood volume (Vc) using combined single-breath DLNO and DLCO measurements. Seventeen SSc patients without pulmonary involvement (SSc), 20 SSc patients with ILD (SSc-ILD), with and without PAH, and 21 healthy controls were included.

Results. DLNO and Dm were reduced in SSc patients as compared with controls, whereas Vc was not significantly different. SSc-ILD patients showed a highly significant decrease in Dm and Vc as compared with SSc patients and controls. Vc tended to be more reduced than Dm in SSc-ILD patients with PAH. Dm and Vc were negatively correlated with PAPs and HRTC scores, but the relationship with the HRTC score was stronger.

Conclusion. DLNO is more sensitive than DLCO in detecting functional impairment in SSc without radiologic or haemodynamic alterations. A disproportional reduction of Dm relative to Vc suggests a thickening of the blood-gas diffusion barrier in these patients. In SSc patients with detectable ILD, the gas exchange impairment is due to both components of lung diffusing capacity, and partitioning of DLCO in Dm and Vc is of little use in distinguishing the patients with only ILD from those with ILD complicated by PAH.

Introduction

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterised by a diffuse endothelial injury of small vessels and immune dysfunction, leading to abnormal fibroblast function and increased extracellular matrix production. SSc is classified into 2 major subtypes by extent of skin sclerosis on physical examination: a limited cutaneous form, with skin thickening confined to the face and extremities, and a diffuse cutaneous form, extended to trunk and proximal limbs. The latter has increased risk of more severe internal organ involvement and worse overall prognosis (1).

Pulmonary involvement, due to interstitial lung disease (ILD) or pulmonary arterial hypertension (PAH), is the leading cause of mortality in patients with SSc (2). ILD occurs in about 60% of patients, most commonly in those with diffuse SSc (3). ILD is characterised by an immune-mediated alveolitis and, at a later stage, pulmonary fibrosis with pathological findings of nonspecific interstitial pneumonia (NSIP) (4). PAH is due to obliterator vasculopathy of pulmonary arterioles that leads to a progressive increase of pulmonary vascular resistance, right ventricular hypertrophy and failure, and death within 2–3 years. The prevalence of PAH in SSc patients has been estimated to be 8–12% (5). Early diagnosis of these conditions requires echocardiography and/or right heart catheterisation and high resolution computer tomography (HRCT), which are expensive and invasive or harmful for the patients due to radiation exposure.

For this reason, pulmonary function tests (PFT) play a key role in early diagnosis and follow-up of pulmonary involvement, as they are acceptable to the patient, quick and easy to perform, and less expensive. In SSc patients with ILD there is a reduction of lung diffusing capacity for carbon monoxide (DLCO) and later a restrictive pattern with decreased forced vital capacity (FVC) (1, 6). In SSc patients with PAH the typical

Key words: nitric oxide, carbon monoxide, interstitial lung diseases, capillary volume, membrane conductance

Received on July 8, 2014; accepted in revised form on November 24, 2014.
Clin Exp Rheumatol 2015; 33 (Suppl. 91): S80-S86.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2015.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2015.
alteration is a reduction of DLCO, without changes in FVC. DLCO is therefore a useful tool in assessment of functional impairment of both ILD and PAH (7). However, DLCO cannot differentiate between ILD and PAH. According to Roughton and Foster’s equation (8), the major determinants of DLCO are the conductance of alveolar-capillary membrane (Dm) and the pulmonary capillary blood volume (Vc). These two components may be affected to a different degree in ILD and PAH. The issue of partitioning DLCO in the assessment of SSc with pulmonary involvement was addressed by two previous studies using the single-breath method at different alveolar oxygen concentrations (9, 10), and one study with simultaneous measurement of lung diffusing capacity for CO (DLCO) and nitric oxide (DLNO) (11), but the results were controversial. Overbeek et al. concluded that partitioned DLCO does not give further information in SSc withILD and PAH (9), whereas Pernet et al. suggested that it might be of interest in identifying ILD in SSc patients (10) and Sivova et al. (11) suggested that calculation of Vc might help in detecting PAH in SSc patients with or without ILD. In the present study, we hypothesised that the determinants of DLCO impairment differ in relationship with interstitial or vascular involvement of the lung of SSc patients. To test this, the diffusing capacity of lung for CO (DLCO) and nitric oxide (DLNO) were measured by the single-breath technique in SSc patients without pulmonary involvement, in SSc patients with ILD and in matched healthy subjects. Dm and Vc were calculated and the values obtained for each group were then compared. Dm and Vc were evaluated in relation to the degree of lung interstitial involvement assessed by HRCT and of vascular involvement assessed by pulmonary artery pressure.

Methods

Study design

Thirty-seven patients attending the outpatient clinic of the Rheumatology Unit of Padova University Hospital were recruited. Inclusion criteria consisted of a definite diagnosis of SSc according to the American Rheumatism Association (ARA) classification (12). Smokers and patients with other lung diseases were not included; patients under treatment with immunosuppressive drugs or with steroids at a daily dosage >10 mg of prednisone were also excluded. A control group of healthy nonsmoking subjects matched for age and gender was enrolled. The study was approved by the University Hospital’s ethics committee (Comitato Etico per la Sperimentazione Azienda Ospedaliera Padova, project approval number 2320P) and informed consent was obtained from all subjects.

All SSc patients underwent HRCT and echocardiography within four months before the study. HRCT was scored according to Strollo et al. (13). The patients with a score ≥5 were classified as affected by parenchymal lung disease (SSc-ILD). Echocardiography was performed by 2-dimensional technique and the Pulmonary Arterial Systolic Pressure (PAPs) was estimated by doppler. The patients were classified as PAH+ if values of PAPs at echocardiogram were >50 mmHg, according to the guidelines of the European Society of Cardiology and European Respiratory Society (14). The subjects with PAPs values between 37 and 50 mmHg were submitted to right heart catheterisation and classified as PAH+ when mean PAP were >25 mmHg.

SSc patients and healthy control subjects performed complete pulmonary function assessment, including spirometry, DLCO and DLNO measurement, Dm and Vc determination.

Pulmonary function tests

DLCO measurement

Forced expiratory flow in 1 s (FEV1), vital capacity (VC), residual volume (RV), total lung capacity (TLC) and DLCO measurement were assessed with a MasterLab Pro (Erich Jaeger GmbH; Höchberg, Germany), according to the recommendations of ATS/ERS (15, 16). The predicted normal values of the Communité Européenne du Carbon et de Acièr (CECA) for volumes (16) and equations of Cotes et al. for DLCO (17) were used.

Statistical analysis

Data are expressed as mean ± SD. The comparison of anthropometric and lung function characteristics among groups was performed with the Kruskal Wallis test and post hoc Mann-Whitney test when appropriate. The relationship between lung function parameters, Dm or Vc and HRCT scores or PAPs was determined using the Spearman rank correlation and multiple regression analysis. A p-value <0.05 was considered significant.

Results

Among the 37 patients included in the study, 17 had a HRCT score ≤5 (0.6±0.8), normal PAH and were in the I WHO functional class (SSc group). The other 20 patients had a HRCT score ≥5 (7.1±2.7); 60% of them were in the II WHO class and the remaining 40%
Lung diffusing capacity in SSc / G. Guarnieri et al.

Table I. Demographic characteristics of the subjects in the 3 groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>SSc</th>
<th>SSc-ILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>8/13</td>
<td>2/15</td>
<td>5/15</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>51 ± 7</td>
<td>49 ± 13</td>
<td>59 ± 13*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 6</td>
<td>163 ± 8*</td>
<td>162 ± 9*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69 ± 14</td>
<td>64 ± 16</td>
<td>68 ± 18</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 4</td>
<td>24 ± 6</td>
<td>26 ± 6</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>–</td>
<td>9 ± 5</td>
<td>12 ± 8</td>
</tr>
</tbody>
</table>

*p vs. SSc, p<0.05; ° vs. Controls, p<0.05

Table II. Static and dynamic lung volumes and lung diffusing capacity for CO in the 3 groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>SSc</th>
<th>SSc-ILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC (% pred.)</td>
<td>94.8 ± 9.5</td>
<td>106.4 ± 16.0*</td>
<td>103.6 ± 22.4</td>
</tr>
<tr>
<td>FEV₁ (% pred.)</td>
<td>102.3 ± 11.0</td>
<td>99.2 ± 0.7</td>
<td>95.0 ± 14.8</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>80.7 ± 5.8</td>
<td>79.3 ± 6.5</td>
<td>72.3 ± 15.7</td>
</tr>
<tr>
<td>TLC (% pred.)</td>
<td>99.3 ± 8.2</td>
<td>96.7 ± 10.6</td>
<td>89.2 ± 15.5</td>
</tr>
<tr>
<td>Alveolar volume (l)</td>
<td>5.7 ± 0.8</td>
<td>4.5 ± 0.7°°</td>
<td>4.0 ± 0.6°°</td>
</tr>
<tr>
<td>DLCO (ml min⁻¹ mmHg⁻¹)</td>
<td>25.2 ± 4.4</td>
<td>20.7 ± 5.9°</td>
<td>14.5 ± 5.8°</td>
</tr>
<tr>
<td>DLCO (% pred.)</td>
<td>97.0 ± 11.2</td>
<td>83.5 ± 16.5°</td>
<td>61.2 ± 19.8°</td>
</tr>
<tr>
<td>KCO (min⁻¹ mmHg⁻¹)</td>
<td>4.5 ± 0.5</td>
<td>4.2 ± 0.8</td>
<td>3.3 ± 0.9°°</td>
</tr>
</tbody>
</table>

*p vs. Controls, p<0.05; ° vs. Controls, p<0.05; °° vs. Controls, p<0.001.

Table III. Lung diffusing capacity for NO (DLNO), alveolar-capillary membrane conductance (Dm) and pulmonary capillary blood volume (Vc) values in the 3 groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>SSc</th>
<th>SSc-ILD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLNO (ml min⁻¹ mmHg⁻¹)</td>
<td>112.1 ± 20.4</td>
<td>90.8 ± 24.9</td>
<td>61.7 ± 26.2</td>
</tr>
<tr>
<td>Dm (ml min⁻¹ mmHg⁻¹)</td>
<td>57.1 ± 10.4</td>
<td>46.2 ± 12.6</td>
<td>31.5 ± 13.5</td>
</tr>
<tr>
<td>Vc (ml)</td>
<td>89.1 ± 11.1</td>
<td>81.0 ± 13.0</td>
<td>61.9 ± 17.3</td>
</tr>
<tr>
<td>Vc (% pred.)</td>
<td>92.4 ± 11.0</td>
<td>83.0 ± 16.0</td>
<td>60.5 ± 17.7</td>
</tr>
</tbody>
</table>

*p vs. Controls, p<0.001.
tial lung disease (HRCT scores) is the major determinant of impairment of Vc and Dm, followed by PAPs (Table IV).

**Discussion**

In this study we demonstrated that a significant reduction in lung diffusing capacity was detectable in SSc patients without evidence of ILD by imaging techniques. The mild impairment of DLCO was mainly due to a decrease in the conductance of alveolar-capillary membrane (Dm), rather than a reduction of pulmonary capillary volume (Vc). When lung involvement was more evident and detectable with HRCT, both components, Dm and Vc, were reduced and contributed to the impairment of DLCO. SSc patients with ILD and PAH had more severe functional alteration than patients with ILD only, and they exhibited a predominant alteration of Vc. However, we showed that the degree of interstitial lung disease was the major determinant of either Dm and Vc, while pulmonary arterial hypertension had a weaker influence on the components of lung diffusing capacity.

The combined single-breath DLNO and DLCO measurement has the advantage that all measurements are obtained in a single maneuver at the same P_atm and cardiac output.

Overbeek *et al.* (9) calculated Dm and Vc using the DLCO single-breath method at different alveolar oxygen concentrations, in SSc patients with radiological signs of ILD, focusing on concomitant PAH. The reduction of both components of diffusing capacity in SSc patients with PAH was consistent with our findings; however, in disagreement with our results, Vc was less affected that Dm. Since the authors were not able to detect a relationship between Dm or Vc and haemodynamic or radiological parameters, the explanation of their results remains undetermined. They concluded that partitioned DLCO is not useful as a screening tool for SSc with ILD and PAH. However, we suggest that evaluation of Dm may reveal pulmonary functional impairment in SSc patients not examined by Overbeek *et al.*, *i.e.*, those without radiological or haemodynamic signs of ILD and PAH, respectively. Minimal HRCT abnormalities (score 1–2) were detected in 8 of the SSc patients without ILD, while in the remaining 9 patients HRCT

![Fig. 1](image1.png)

**Fig. 1.** (a) Alveolar-capillary membrane conductance (Dm) and (b) pulmonary capillary blood volume (Vc) in patients with systemic sclerosis associated with parenchymal lung disease without pulmonary arterial hypertension (SSc-ILD PAH-) and with pulmonary arterial hypertension (SSc-ILD PAH+). Black bars represent medians.

![Fig. 2](image2.png)

**Fig. 2.** Correlation between DLCO and PAPs (a), or HRCT scores (b) in the 37 patients with systemic sclerosis. Black circles: SSc patients; open circles: SSc-ILD patients.
was fully negative. Dm in the former subgroup tended to be lower than in the latter, indicating that morphological alterations, albeit insufficient to diagnose an ILD, may have functional consequences. A similar group of SSc patients without ILD and PAH was investigated recently with the combined DLCO/DLNO measurements, but it remains undetermined whether there is agreement with our findings due to the lack of a control group of healthy subjects in the study design (11).

Pernot et al. (10) used the single-breath method at different alveolar oxygen concentrations for partitioning DLCO in SSc patients with and without ILD, all with normal PAH. They showed a significant reduction of DLCO and Dm in SSc-ILD+ patients compared with SSc-ILD-. Since it was shown that Dm/Vc was more sensitive and specific than any of the other pulmonary function tests for identifying ILD, they suggested that the partitioning of DLCO might be of interest for the detection of ILD in SSc. However, some of the findings are surprising because Vc resulted higher in SSc with ILD than in SSc with normal HRCT. The authors speculated that an association between low Dm and high Vc could be found in the case of high pulmonary venous pressure such as in pulmonary vein compression, but there was no evidence for this in ILD associated with SSc. Indeed, Dm and Vc contributed almost equally to DLCO reduction in a population of ILD due to idiopathic interstitial pneumonia evaluated with the combined DLCO/DLNO method. Other studies that used the DLCO/DLNO method in various forms of ILD demonstrated a consistent decrease in both Dm and Vc (22-25). Van der Lee et al. (22), showed that Vc and Dm were 63% and 53% of predicted value, respectively, indicating an impairment of both components of DLCO with slight prevalence of Dm in ILD of various aetiologies. In patients with sarcoidosis, Dm and Vc were significantly reduced compared with normal subjects (average 41% and 57%, respectively) (23).

In contrast, another study on pulmonary sarcoidosis found reduced DLCO associated with severely decreased Dm (24). Vc was unchanged, when the components of lung diffusing capacity were calculated performing DLCO at two alveolar $O_2$ tensions according to the original Roughton-Forster method (24). Using the same method, the study of Bonay et al. (24) on chronic infiltrative lung disease detected a reduction of DLCO and Dm, but values of Vc (presented as percentage predicted) were substantially normal or slightly impaired. However, none of the studies observed an increase in Vc in ILD.

The inconsistency in the results obtained in different studies may be due to some differences in underlining pathological
The small number of patients involved in this study might be considered a shortcoming. However, the size of our sample is in line with previous investigations of SSc (9-11). It should also be remembered that SSc is not a frequent disease; in fact, a prevalence of between 56 and 341 cases of SSc per million has been estimated for recent years (30). Application of this information in clinical practice would need to know when an excessive decline in DLNO occurs in a single patient, but longitudinal investigation has not been performed yet. Based on equation of reference values in adult population, DLNO shows a decline of 1.21 ml/min/mmHg per year (19). However, this estimate is based on a cross sectional study and is referred to healthy subjects.

The DLCO/DLNO method is based on some assumptions that are still controversial. We assumed that $\theta_{NO}$ is negligible, since $\theta_{NO}$ is greater than $\theta_{CO}$. However, the true value of $\theta_{NO}$ remains uncertain. We used a ratio DmNO/DmCO of 1.97 based on solubility coefficient of NO in water, while NO solubility in tissue or plasma is not necessarily the same. However, these potential errors in assumed constants are systematic and unlikely to significantly bias the differences detected among groups of patients.

In conclusion, the results suggest that DLNO is more sensitive than DLCO in detecting functional impairment in SSc patients without any radiologic or haemodynamic alterations. In these patients, a disproportional reduction of Dm relative to Vc suggests that thickening of the blood-gas diffusion barrier contributes to the gas transport limitation. When ILD in SSc patients becomes evident to HRCT, the impairment of gas exchange is due both to components of lung diffusing capacity and to partitioning of DLCO in Dm, and Vc is of little use in distinguishing patients with ILD only from those with ILD complicated by PAH. Longitudinal studies may be helpful to understand better whether measurements of Dm and Vc are able to predict the outcome of the disease in SSc patients.

### Table IV. Multiple regression models of DLCO, DM and Vc with HRCT scores, PAPs and alveolar volume (VA).

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent variables</th>
<th>R</th>
<th>Regression coefficients (SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLCO</td>
<td>HRCT scores</td>
<td>0.54</td>
<td>-0.34 (0.26)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>PAPs</td>
<td></td>
<td>-0.15 (0.06)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td></td>
<td>1.69 (1.28)</td>
<td>NS</td>
</tr>
<tr>
<td>Dm</td>
<td>HRCT scores</td>
<td>0.61</td>
<td>-1.81 (0.55)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>PAPs</td>
<td></td>
<td>-0.32 (0.14)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td></td>
<td>0.29 (2.69)</td>
<td>NS</td>
</tr>
<tr>
<td>Vc</td>
<td>HRCT scores</td>
<td>0.63</td>
<td>-2.07 (0.65)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>PAPs</td>
<td></td>
<td>-0.41 (0.16)</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>VA</td>
<td></td>
<td>1.0 (3.41)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Acknowledgments
The authors wish to thank Luigi Zedda, BSc, for his technical assistance and Kathleen Parker, Med, for editing the manuscript.

References