Lung capillary blood volume and membrane diffusion in idiopathic interstitial pneumonia

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KEYWORDS
Capillary blood volume; DLCO/DLNO; Idiopathic interstitial pneumonia; Membrane conductance; Pulmonary arterial pressure

Summary
Rationale: Diffusing capacity of the lung for carbon monoxide (DLCO) is a good marker of disease severity in patients with idiopathic interstitial pneumonia (IIP). The combined diffusing capacity of nitric oxide (DLNO) and DLCO determines the two components of diffusion: membrane conductance (Dm, CO) and pulmonary capillary blood volume (Vc).
Objectives: The aim of this study was to evaluate Vc and Dm, CO in patients with fibrosing IIP in order to determine the relative contribution of membrane resistance and vascular resistance to the loss of DLCO.
Methods: 32 patients with IIP (IPF: n = 22, NSIP: n = 10) were evaluated using MRC dyspnea scale, plethysmography, combined DLNO/DLCO, 6-min walk test (6 MWT), echocardiography and chest computed tomography (chest CT).
Results: DLCO (41.8 ± 11.9%pred), Dm, CO (40.5 ± 12.7%pred) and Vc (41.9 ± 18%pred) were severely and equally reduced. Dm, CO and Vc were related to MRC scale, FVC, maximal desaturation during 6 MWT, and systolic pulmonary artery pressure (sPAP). There was no correlation with the extent of fibrotic changes on chest CT.
Conclusions: Our main results indicate that Dm, CO and Vc contribute almost equally to DLCO reduction in IIP. Dm, CO and Vc are related to functional indicators of disease severity and to sPAP in agreement with the concept of vascular involvement in IIP.
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Introduction

Idiopathic pulmonary fibrosis (IPF) and non-specific interstitial pneumonia (NSIP) are two idiopathic interstitial pneumonias (IIP) of unknown etiology and poor prognosis. The pathogenesis of lung fibrosis is not clearly understood, but alveolar epithelial cell injury, dysregulation of fibroblasts, vascular injury and aberrant angiogenesis related to vascular remodeling are key elements.

Diffusing capacity of the lung for carbon monoxide (DLCO) is a simple test which evaluates the efficiency of pulmonary gas exchange. It is a valuable tool in the assessment of pulmonary diseases and particularly IPF. Alteration of DLCO may result from changes in gas exchange area, alveolar capillary membrane thickness and ventilation/perfusion relationship in the lung. Since the pulmonary capillary bed also takes part in the gas exchange area, DLCO may also reflect involvement of pulmonary capillaries.

According to the model of Roughton and Forster, DLCO is composed of two resistances arranged in series: \( \frac{1}{\text{DLCO}} = \frac{1}{Dm} + \frac{1}{Vc} \). Pulmonary membrane diffusing capacity (Dm) for carbon monoxide (Dm, CO) is the CO conductance across the alveolar-capillary tissue membrane and plasma barrier; \( vCO \) is the rate of carbon monoxide uptake by whole blood and combination with Hb measured in vitro; Vc is the pulmonary capillary blood volume. The measurement of diffusing capacity of the lung using the transfer gas nitric oxide (NO) and CO together permits to obtain Dm, CO and Vc in a single-breath experiment.

The aim of this study was first to measure Vc and Dm, CO in patients with fibrosing IIP in order to determine the relative contribution of membrane resistance and vascular resistance in the loss of DLCO and secondly to study their relations with pulmonary function tests, chest computed tomography (chest CT) and echocardiography.

Patients and methods

Thirty two patients with IIP were included in this prospective study. Inclusion criteria consisted of diagnosis of IPF according to the American Thoracic Society/European Respiratory Society guidelines and/or histopathological evidence for usual interstitial pneumonia, or diagnosis of NSIP (radiographic or histopathological diagnosis). Patients were not included if they had another pulmonary disease (including obstructive disease), left heart failure or a history of pulmonary embolism. Connective tissue diseases were ruled out. No acute exacerbation was observed in the three months preceding inclusion. All patients completed pulmonary function tests. Twenty seven patients completed a modified MRC dyspnea scale. An informed consent was obtained from all patients and approval for the use of these data was provided by the Institutional Review Board of the French learned society for respiratory medicine.

Pulmonary function tests

Resting pulmonary function tests included measurement of lung volumes by plethysmography and single breath diffusion capacity of the lung for carbon monoxide (DLCO) (Jaeger-Masterlab, Belgium) as described by Aguilaniu et al. The measurements of DLNO/DLCO were accepted if two successive measurements of DLNO and DLCO gave figures within 10%, otherwise a third measurement was performed. Values of Dm, CO and Vc were calculated according to the model of Guénard et al. Hemoglobin concentration was not measured but set at 13.5 g/dL for women and 14.5 g/dL for men. \( vCO \) values were multiplied by 13.5/14.9 and 14.5/14.9 respectively. Reference values for DLNO, Dm, CO and Vc used were those established by Aguilaniu et al.

Transthoracic echocardiography

A transthoracic echocardiography was performed at rest, with the patient lying on the left side. Resting measurements included left ventricular diameter and volume as well as left ventricular ejection fraction using the biplane Simpson method. Characteristic of the right ventricle (RV) was also assessed namely, the RV end-diastolic diameter and shortening fraction, tricuspid annular plane systolic excursion (TAPSE), and tricuspid S-waves on Doppler tissue imaging also provided an appreciation of the RV systolic function. We also analysed pulmonary acceleration time, the right ventricular-right atrium gradient on continuous Doppler scans by analysis TR flow, subaortic time-velocity integral of flow and inferior vena cava collapsibility to estimate pressure in the RA. Systolic pulmonary arterial pressure (sPAP) is considered equal to right ventricular systolic pressure in the absence of pulmonary valve stenosis. Right ventricular systolic pressure, and so sPAP can be estimated using continuous wave Doppler.

Chest computed tomography

Chest CT examinations were reviewed by two experienced radiologists unaware of lung function data. Scans were evaluated using a thin section CT (HRCT) scoring system for lung fibrosis, based on ground glass attenuation (HRCT alveolar score) and fibrotic change (HRCT interstitial score) previously described.

Statistical analysis

Results are presented as mean ± SD. Following ATS/ERS 2005 guidelines, the lower limits of normal (LLN) were set at the level of 5th percentile (or mean minus 1.645 RSD) of
each reference population. Results were conventionally expressed as percent predicted. All statistical analysis were carried out with GraphPad Prism 4.0 software (San Diego, California, USA). Correlations were analysed using Spearman’s rank correlation test: three variables were tested (DLCO, Vc and Dm, CO). For each variable a correlation with FVC, minimal saturation during 6 MWT, sPAP and MRC was studied. Four tests being performed with each variable, a Bonferroni correction was performed to limit type I errors. The corresponding level of significance is thus set to 0.0125 (0.05/4).

Results

Characteristics of the population are summarized in Table 1. The overall population consisted of 25 men and 7 women with a mean age of 63.1 ± 10.4 years. Twenty two patients had a diagnosis of IPF and 10 of NSIP. Diagnosis was obtained by open lung biopsy in 8 cases. At the time of inclusion in the study, 9 patients received steroids, 2 patient received azathioprine, 3 patients received mycophenolate mofetil, 1 patient received methotrexate, 11 were included in therapeutical trials and 10 were untreated.

Pulmonary function tests and echocardiography

Results of pulmonary function tests are summarized in Table 1: 25 of the 28 patients who underwent plethysmography demonstrated a restriction (TLC < LLN). DLCO was below LLN for all patients. Sixteen of the 31 (51.6%) patients who underwent echocardiography had an estimated sPAP higher than 35 mmHg.

Chest computed tomography

Thirty two chest CT examinations were reviewed in total: 17 scans were reviewed on films, and the rest interpreted on CD-ROM. Eighteen scans were reviewed using 5 mm cuts, 1 using 3 mm cuts, one using 7 mm cuts and 10 using 1 mm cuts, though 1 mm cuts every 10 mm (old HRCT protocol) were reviewed in each case. Thirteen studies were performed using intravenous contrast medium.

An overall average ground glass score of 2.31 and an interstitial score of 1.69 were obtained for the group of patients studied. The highest ground glass and interstitial scores were noted in the lower lobes with the lowest scores in the upper lobes, as could be expected, given the cases involved patients with diagnoses of IPF and NSIP. The left lung values overall, were greater than those for the right lung, concerning both ground glass and interstitial elements; left lower lobe interstitial change average score was slightly greater than that for the right lower lobe.

Correlations between Vc, Dm, CO and studied parameters

Correlations are illustrated by Figs. 2–4. DLCO, Dm, CO and Vc were related to FVC (respectively r = 0.63 p = 0.0001; r = 0.56 p = 0.0008; and r = 0.56 p = 0.0008). DLCO expressed as percent of predicted values (DLCO%), Dm, CO % and Vc% were also related to FVC% (Fig. 2). DLCO, Dm, CO and Vc were related to the MRC score (respectively r = −0.47 p = 0.01; r = −0.45 p = 0.01 and r = −0.39 p = 0.008).

DLCO was related to the 6 MWT minimal SpO2. So were Dm, CO and Vc (Fig. 3). Lastly, DLCO was related to sPAP and the same relation was found with Dm, CO and Vc (Fig. 4).

No relations were observed between DLCO, Dm, CO, Vc and CT scores, neither with ground glass score nor with interstitial score.

Discussion

To our knowledge, this is the first study evaluating the clinical relevance of combined DLCO/DLNO method in patients with IIP. There were three main findings: 1/Dm, CO and Vc contribute almost equally to DLCO reduction in IIP; 2/Dm, CO and Vc are related to several pulmonary

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the 32 patients.</th>
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<tbody>
<tr>
<td></td>
<td>Mean ± SD % Predicted</td>
</tr>
<tr>
<td>Age (years)a</td>
<td>63.1 ± 10.4</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>25/7</td>
</tr>
<tr>
<td>height (m)b</td>
<td>1.7 ± 0.08</td>
</tr>
<tr>
<td>Weight (kg)b</td>
<td>83.2 ± 16</td>
</tr>
<tr>
<td>MRC (1/4)e</td>
<td>1.4 ± 1</td>
</tr>
<tr>
<td>FVC (L)a</td>
<td>2.73 ± 0.76 75.7 ± 17.1</td>
</tr>
<tr>
<td>FEV 1 (L)a</td>
<td>2.15 ± 0.56 75.5 ± 16.6</td>
</tr>
<tr>
<td>TLC (L)d</td>
<td>4.28 ± 0.87 69 ± 11.6</td>
</tr>
<tr>
<td>DLCO 10 s (mL/mmHg/Ha)</td>
<td>10.7 ± 3.35 41.8 ± 11.9</td>
</tr>
<tr>
<td>DLCO/VA (mL/min/mmHg/L)a</td>
<td>2.9 ± 0.76 71.4 ± 16.2</td>
</tr>
<tr>
<td>6 MWT : distance (m)b</td>
<td>391.8 ± 108.1</td>
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<tr>
<td>6 MWT : minimal saturation (%)b</td>
<td>87.4 ± 5</td>
</tr>
<tr>
<td>sPAP (mmHg)c</td>
<td>33.8 ± 8.3</td>
</tr>
<tr>
<td>DLCO 4 s (mL/mmHg/min)a</td>
<td>10 ± 3.6 40.3 ± 15.1</td>
</tr>
<tr>
<td>DLNO (mL/mmHg/min)a</td>
<td>56.9 ± 18.33 40.5 ± 12.7</td>
</tr>
<tr>
<td>Dm, CO (mL/mmHg/min)a</td>
<td>28.9 ± 9.3 40.5 ± 12.7</td>
</tr>
<tr>
<td>Vc (mLb)</td>
<td>27.5 ± 10.78 41.9 ± 18</td>
</tr>
</tbody>
</table>

MRC: Medical Research Council dyspnea scale; DLCO: Diffusing capacity of the lung for carbon monoxide; Vc: capillary blood volume; Dm, CO: membrane conductance for carbon monoxide; 6 MWT: 6 min walk test; sPAP: systolic pulmonary arterial pressure.
shown that the Dm, CO component was the principal exacerbation. Reduction of Dm may be caused by destruction, obstruction or compression of capillaries. Those mechanisms are observed in fibrotic lung: capillary density is decreased in the most extensively fibrotic regions; small clots were detected in the alveolar capillary bed in lungs of patients who died after an acute exacerbation. Reduction of Dm may be caused by destruction, thickening or infiltration of the membrane. The extent of these changes increases with disease progression. It is therefore interesting to note that both Dm, CO and Vc are correlated with classical functional indicators of disease severity and progression. It is therefore interesting to note that both Dm, CO and Vc are correlated with classical functional indicators of disease severity and progression of active fibrosis and increased vessel density in areas of mild interstitial change. This heterogeneity, paired with anastomoses between the pulmonary and systemic circulation could lead to shunting of blood away from areas of gas exchange and thus to ventilation/perfusion mismatch.

In our population, DLCO, Vc and Dm were severely and equally reduced. In patients with ILD, reduction of DLCO may result from changes in gas exchange area, barrier thickness and ventilation/perfusion mismatching of the lung. Reduction of Vc may be caused by destruction, obstruction or compression of capillaries. Those mechanisms are observed in fibrotic lung: capillary density is decreased in the most extensively fibrotic regions; small clots were detected in the alveolar capillary bed in lungs of patients who died after an acute exacerbation. Reduction of Dm may be caused by destruction, thickening or infiltration of the membrane. The extent of these changes increases with disease progression. It is therefore interesting to note that both Dm, CO and Vc are correlated with classical functional indicators of disease severity and progression. In the study by Van der Lee et al., DLCO was found to be 65% of predicted value, Vc 63% and Dm 53%, in diffuse parenchymal lung disease of various aetiologies, suggesting that Dm, CO was again the major determinant of DLCO reduction. Our finding is of importance because we only included patients with IPF or NSIP. This result suggests that the vascular component is more affected in IIP than in other ILD. In IPF, the degree of ventilation/perfusion mismatch is greater than it is seen in other fibrotic lung diseases, even after adjusting for disease severity. This could be explained by heterogeneity of interstitial microvascularity reported in IPF with vascular regression in sites of active fibrosis and increased vessel density in areas of mild interstitial change. This heterogeneity, paired with anastomoses between the pulmonary and systemic circulation could lead to shunting of blood away from areas of gas exchange and thus to ventilation/perfusion mismatch.

This result could also suggest that in IPF, Vc and Dm, CO are dependent. This idea could be illustrated by the high correlation found between the two components in our study. Some investigations in patients with IPF show that capillary density is significantly decreased in diseased areas, leading to a decrease in the Vc component of the DLCO in addition to the already lowered Dm, CO component as a consequence of the diseased-thickened membranes, thus making the Vc component dependent on the Dm, CO component. Besides, hypoxemia due to thickened membranes can lead to pulmonary vasoconstriction. Moreover, the decrease of Vc will result in a reduction in surface area available for gas exchange and therefore in a decrease of Dm. NO diffusion might also be impaired by ventilation heterogeneity, a known mechanism of diffusion limitation. If Dm, CO and Vc are dependent, the separation of the DLCO in these two components becomes clinically irrelevant. Nevertheless, since Dm, CO and Vc can be affected in different ways in various diseases, their parallel impairment in IPF/NSIP remains interesting. Another area of uncertainty regarding the DLNO technique is the value of r(NO). A recent study by Borland et al. suggests that blood conductance for NO might not be infinite, due to limiting factors of red blood cells.

functional tests parameters; 3/Both Dm, CO and Vc are correlated to sPAP.

Dm, CO and Vc contribute almost equally to DLCO reduction in IIP and are related to functional indicators of disease severity

In our population, DLCO, Vc and Dm were severely and equally reduced. In patients with ILD, reduction of DLCO may result from changes in gas exchange area, barrier thickness and ventilation/perfusion mismatching of the lung. Reduction of Vc may be caused by destruction, obstruction or compression of capillaries. Those mechanisms are observed in fibrotic lung: capillary density is decreased in the most extensively fibrotic regions; small clots were detected in the alveolar capillary bed in lungs of patients who died after an acute exacerbation. Reduction of Dm may be caused by destruction, thickening or infiltration of the membrane. The extent of these changes increases with disease progression. It is therefore interesting to note that both Dm, CO and Vc are correlated with classical functional indicators of disease severity and progression. In our study, we tried to determine whether the membrane resistance or the vascular resistance accounted for the loss of DLCO. Clearly, it shows that both the vascular component and the membrane component are severely and equally reduced: the disruption in the membrane and the reduction of capillary blood volume contribute equally to the abnormal diffusing capacity in IIP.

To our knowledge, this is the only study in interstitial lung disease (ILD) to suggest these components are affected equally. Other studies in various forms of ILD have demonstrated a consistent decrease in Dm, CO with relative preservation of Vc. In a previous study using the Roughton and Forster method in patients with pulmonary sarcoidosis, the authors have shown that Dm, CO was the major determinant of impaired DLCO. Overbeek et al has shown that the Dm, CO component was the principal contributor to the reduction in DLCO in systemic sclerosis with or without Pulmonary Arterial Hypertension (PAH). In the study by Van der Lee et al., DLCO was found to be 65% of predicted value, Vc 63% and Dm 53%, in diffuse parenchymal lung disease of various aetiologies, suggesting that Dm, CO was again the major determinant of DLCO reduction. Our finding is of importance because we only included patients with IPF or NSIP. This result suggests that the vascular component is more affected in IIP than in other ILD. In IPF, the degree of ventilation/perfusion mismatch is greater than it is seen in other fibrotic lung diseases, even after adjusting for disease severity. This could be explained by heterogeneity of interstitial microvascularity reported in IPF with vascular regression in sites of active fibrosis and increased vessel density in areas of mild interstitial change. This heterogeneity, paired with anastomoses between the pulmonary and systemic circulation could lead to shunting of blood away from areas of gas exchange and thus to ventilation/perfusion mismatch.

Figure 1 A: Relationship between Dm, CO and Vc B: Relationship between Dm, CO and Vc expressed as percent of predicted values Vc: capillary blood volume Dm (Dm, CO): Membrane conductance for carbon monoxide r: Spearman coefficient.
Pulmonary hypertension (PH) is a severe complication of IIP, associated with a poor prognosis. Significant PH is more frequent when underlying fibrosis is severe, but may occur at any stage of the disease process. In our study, we estimated the sPAP values by transthoracic echocardiography coupled with Doppler, which is a non-invasive technique. sPAP estimated this way correlated well with the values measured in patients with right cardiac catheterization. However, we must point out that some authors reported various ranges of discordant results between echocardiography and right heart catheterization. Care must be taken to align the Doppler beam with the direction of the flow or underestimation of the pulmonary artery pressure may result. With these caveats, Doppler echocardiography is a convenient, non-invasive, and relatively accurate tool for the evaluation of PH.

In IPF, DLCO is lower in patients with PH on right heart catheterization. Hamada showed a negative correlation between DLCO (expressed in % of predicted values) and mPAP. In our study we found this correlation between DLCO and sPAP. However, Nathan et al. demonstrate that DLCO levels measured in isolation are not reliably indicative
In our study, sPAP correlated inversely with Vc and Dm, CO. Relations between hemodynamic values and the vascular component of DLCO have been previously reported: Oppenheimer et al. showed an inverse relationship between mPAP and Vc in a group with miscellaneous forms of PH, whereas they did not find the correlation between Dm, CO and mPAP. Others found no relationship between hemodynamic parameters of pulmonary hypertension and Dm, CO or Vc in systemic sclerosis. Borland et al. found that Vc was lower in patients with severe unexplained pulmonary hypertension than in normal subjects. Another study, considering patients with various infiltrative lung disease (connective tissue disease, asbestosis, sarcoidosis or unknown origin) showed that Vc, determined by the Roughton and Forster method was paradoxically often high, almost normal in patients with chronic infiltrative lung disease and PH. To our knowledge, the present study is the first to demonstrate a relationship between sPAP and both Dm, CO and Vc in fibrosing IIP.

The recent data from clinical studies suggest that the predominant mechanisms for the development of PH in IPF may not be hypoxic vasoconstriction and pulmonary capillary loss. The presence of PH cannot be explained in all patients by hypoxemia or degree of lung function reduction. It seems likely that the biological processes underlying fibrosis progression are also involved in the vascular remodeling and PH. The histopathologic changes in pulmonary arteries of UIP lungs show thickening of the smooth muscle layer, proliferative intima lesions and complete occlusion of the vessel by scar tissue and plexiform lesions. It is therefore not surprising that both the membrane and the vascular resistance are related to the increase of sPAP.

In conclusion, our results demonstrate that alterations of Dm, CO and Vc contribute equally to reduction of DLCO in patients with IIP. They are both related to severity of lung involvement and to the development of increased pulmonary arterial pressure. Due to the parallel impairment of membrane and vascular component in IIP, DLCO-DLNO appears at present to add little in the routine assessment of these disorders, clinical correlates being in addition similar for DLCO, Dm and Vc. However the relevance of DLNO/DLCO to evaluate new therapeutic interventions targeting the vascular component should be studied.

Conflict of interest

All authors have read the manuscript and declare no potential conflict of interest, no prior publication or concurrent submission and no copyright constraints.

References
