Lung diffusing capacity for nitric oxide as a marker of fibrotic changes in idiopathic interstitial pneumonias

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Barisione G, Brusasco C, Garlaschi A, Baroffio M, Brusasco V. Lung diffusing capacity for nitric oxide (DLNO) was better reflects fibrotic changes than DLCO. DLNO-DLCO were measured simultaneously in 30 patients with UIP-IPF and 30 with NSIP. Eighty-one matched healthy subjects served as a control group. The amount of pulmonary fibrosis was estimated by CT volumetric analysis of visually bounded areas showing reticular opacities and honeycombing. DMCO and pulmonary capillary volume (VC) were calculated. DLNO was below the lower limit of normal in all UIP-IPF and NSIP patients and significantly correlated with fibrosis extent in both diseases, whereas VC was weakly correlated with fibrosis in UIP-IPF and uncorrelated in NSIP, with normal values in half of patients. In conclusion, measurement of DLNO may provide a more sensitive evaluation of fibrotic changes than DLCO in either UIP-IPF or NSIP, because it better reflects DMCO.

NEW & NOTEWORTHY

Lung diffusing capacity for CO (DLCO) is decreased in both usual interstitial pneumonia-idiopathic pulmonary fibrosis (UIP-IPF) and nonspecific interstitial pneumonia (NSIP), but is moderately related to computed tomography (CT)-determined fibrotic changes. This may be due to the relative insensitivity of DLCO to changes in alveolar membrane diffusive conductance (DMCO). The purpose of this study was to determine whether measurement of lung diffusing capacity for nitric oxide (DLNO) better reflects fibrotic changes than DLCO. DLNO-DLCO were measured simultaneously in 30 patients with UIP-IPF and 30 with NSIP. Eighty-one matched healthy subjects served as a control group. The amount of pulmonary fibrosis was estimated by CT volumetric analysis of visually bounded areas showing reticular opacities and honeycombing. DMCO and pulmonary capillary volume (VC) were calculated. DLNO was below the lower limit of normal in all patients irrespective of extent and nature of disease, whereas DLCO was within the normal range in a nonnegligible number of patients. Both DLNO and DLCO were significantly correlated with visual assessment of fibrosis but DLNO more closely than DLCO. DMCO was also below the lower limit of normal in all UIP-IPF and NSIP patients and significantly correlated with fibrosis extent in both diseases, whereas VC was weakly correlated with fibrosis in UIP-IPF and uncorrelated in NSIP, with normal values in half of patients. In conclusion, measurement of DLNO may provide a more sensitive evaluation of fibrotic changes than DLCO in either UIP-IPF or NSIP, because it better reflects DMCO.

usual interstitial pneumonia; nonspecific interstitial pneumonia; computed tomography; alveolar membrane diffusive conductance; pulmonary capillary volume

USUAL interstitial pneumonia-idiopathic pulmonary fibrosis (UIP-IPF) and nonspecific interstitial pneumonia (NSIP) are diffuse inflammatory and fibrotic lung diseases conventionally grouped under the term of idiopathic interstitial pneumonias (IIPs) (45). The differentiation between UIP-IPF and NSIP is based on chest computed tomography (CT), with prevalence of reticular opacities and honeycombing (RO-HC) in the former and ground glass attenuation (GGA) in the latter (45). Lung function studies in IIPs generally show a restrictive disorder with decreased lung diffusing capacity for carbon monoxide (DLCO) (30) and are deemed to be useful for patients staging and prognosis (40) but not for differentiating UIP-IPF from NSIP.

The functional abnormalities of IIPs are consistent with loss of alveolar surface area and thickening of air-to-blood barrier (23) although a reduced DLCO may also reflect changes in lung diffusing capacity (42). Indeed, previous studies have shown the DLCO decrement to be moderately correlated with the extent of fibrotic changes on CT scan (5, 27, 49). Because the limit to CO transport is by ~80% due to its reaction rate with blood hemoglobin (Hb) (34), DLCO is relatively insensitive to changes in alveolar membrane diffusive conductance (DMCO). Unlike CO, nitric oxide (NO) has a much faster rate of reaction with blood hemoglobin (34), DLNO can be regarded as a more sensitive measurement of gas transport across the alveolar membrane (9, 22). Moreover, simultaneous measurements of dual DLNO and DLCO (DLNO-DLCO) have been proposed to separate the contributions of DMCO and pulmonary capillary volume (VC) to gas transfer (9, 22). By using this approach, two studies have suggested that DMCO and VC are greatly and equally reduced in IIPs (46, 50). One study (50) also investigated the relationships between DLCO components and CT findings and failed to show significant correlations between DMCO or VC decrement and the extent of RO-HC or GGA. One possible reason for this unexpectedly negative finding is that the amount of RO-HC, which is supposedly the major reason for diffusion limitation in IIPs, was small compared with the extent of GGA.

The present study was undertaken to test the hypothesis that the measurement of DLNO may provide an assessment of the severity of fibrotic process in IIPs more sensitive than DLCO and whether differences exist between UIP-IPF and NSIP due to a different impairment of DMCO or VC.

METHODS

Subjects. We used retrospectively collected data of 60 Caucasian patients with clinical and radiological features of IIPs referred to Respiratory Pathophysiology Unit for a complete pulmonary func-
Lung Diffusing Capacity for NO in Idiopathic Interstitial Pneumonias  •  Barisone G et al.

Table 1. Subjects’ anthropometric data and standard lung function parameters

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n = 81$)</th>
<th>UIP-IPF ($n = 30$)</th>
<th>NSIP ($n = 30$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>46/35</td>
<td>18/12</td>
<td>20/10</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>68 ± 9 [29, 75]</td>
<td>72 ± 7 [53, 95]</td>
<td>67 ± 12 [37, 89]</td>
<td>0.05</td>
</tr>
<tr>
<td>Stature, m</td>
<td>1.64 ± 0.09 [1.46, 1.87]</td>
<td>1.64 ± 0.11 [1.44, 1.82]</td>
<td>1.65 ± 0.08 [1.50, 1.83]</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25 ± 2 [22, 31]</td>
<td>27 ± 3 [20, 33]</td>
<td>26 ± 2 [21, 28]</td>
<td>0.15</td>
</tr>
<tr>
<td>Current/former/never smoker</td>
<td>0/31/50</td>
<td>5/14/11</td>
<td>5/16/0</td>
<td></td>
</tr>
<tr>
<td>FVC, liters</td>
<td>3.64 ± 0.86 [2.75, 5.80]*</td>
<td>2.15 ± 0.71 [1.10, 4.08]**†</td>
<td>2.86 ± 0.92 [1.30, 4.78]**†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>119 ± 14*</td>
<td>75 ± 22*</td>
<td>90 ± 27*</td>
<td></td>
</tr>
<tr>
<td>FEV₁, liters</td>
<td>2.64 ± 0.66 [2.11, 4.19]*</td>
<td>1.77 ± 0.52 [0.92, 2.88]**‡</td>
<td>2.21 ± 0.70 [1.07, 3.71]**‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>110 ± 12*</td>
<td>80 ± 23*</td>
<td>89 ± 24*</td>
<td></td>
</tr>
<tr>
<td>TLC, liters</td>
<td>5.86 ± 1.20 [4.45, 8.06]*</td>
<td>3.58 ± 1.08 [2.31, 6.02]**‡</td>
<td>4.56 ± 1.21 [2.03, 7.00]**‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>103 ± 6*</td>
<td>63 ± 14**</td>
<td>80 ± 20**</td>
<td></td>
</tr>
<tr>
<td>DLCO,stand, ml·min⁻¹·mmHg⁻¹⁻¹</td>
<td>22.3 ± 4.91 [17.2, 37.6]*</td>
<td>9.92 ± 3.56 [4.70, 18.8]*</td>
<td>14.7 ± 4.36 [5.60, 25.6]*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>97 ± 8*</td>
<td>46 ± 15*</td>
<td>62 ± 15*</td>
<td></td>
</tr>
<tr>
<td>DLCO,stand·VA, ml·min⁻¹·mmHg⁻¹⁻¹⁻¹</td>
<td>4.09 ± 0.42 [3.36, 5.29]*</td>
<td>3.14 ± 0.97 [1.51, 5.88]*</td>
<td>3.53 ± 0.79 [2.19, 5.42]*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% predicted</td>
<td>101 ± 10*</td>
<td>79 ± 24*</td>
<td>82 ± 18*</td>
<td></td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>ND</td>
<td>71.6 ± 12.3 [42.5, 88.8]</td>
<td>77.0 ± 13.4 [43.9, 97.7]</td>
<td>0.07</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>ND</td>
<td>39.8 ± 3.28 [34.4, 45.7]</td>
<td>38.7 ± 3.45 [32.0, 47.3]</td>
<td>0.27</td>
</tr>
<tr>
<td>P(A-a)O₂, mmHg</td>
<td>ND</td>
<td>27.6 ± 13.0 [3.72, 58.3]</td>
<td>23.6 ± 12.9 [4.69, 51.7]</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are absolute numbers or means ± SD [range]. UIP-IPF, usual interstitial pneumonia-idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; FVC, forced vital capacity; FEV₁, forced expiratory volume in one second; TLC, total lung capacity; DLCO, standard single-breath lung diffusing capacity for carbon monoxide (CO); VA, alveolar volume; PaO₂ and PaCO₂, partial pressures of oxygen (O₂) and carbon dioxide (CO₂) in arterial blood; P(A-a)O₂, alveolar-arterial O₂ tension difference; ND, not done. Symbols indicate statistically significant ($P < 0.001$) differences versus controls (*) and between groups (‡) by ANOVA and Holm-Sidak or Dunn’s method and unpaired Student’s $t$-test whenever appropriate.

Fig. 1. Manual segmentation image taken 1 cm above the dome of the right hemidiaphragm, i.e., at 5th axial level, of a nonenhanced computed tomography (CT) scan obtained in supine position from a representative usual interstitial pneumonia-idiopathic pulmonary fibrosis (UIP-IPF) patient included in the current study. Normal parenchyma is bounded by continuous white lines, ground glass by continuous black lines, and reticular opacities-honeycombing areas by dashed white lines. See text for further details.
The washout volume was reduced to 500 ml (33). The values of \( \text{NO} \cdot \text{ml blood} \) at 37.5°C, respectively (35). When a finite and mean value of two properly performed maneuvers was retained for (DMCO, 7.66 and VC, 7.66, respectively) as follows:

\[
\text{DMCO,}\, \text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} = \frac{1}{\text{VA/TLCpleth}} \cdot 0.94
\]

\[
\text{VC,}\, \text{ml} = 55.5 \pm 10.9^+ \quad 17.9 \pm 6.39^+ \quad 28.8 \pm 11.1^+ < 0.001
\]

\[
\text{VA}/\text{Hb}, \, \text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} = 8.62 \pm 3.60^* \quad 3.17 \pm 1.01^+ < 0.001
\]

\[
\text{VA}/\text{Hbmeas}, \, \text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1} = 4.26 \pm 0.80^* \quad 4.38 \pm 0.80^+ < 0.001
\]

\[
\text{NO}/(\frac{d}{z}) \cdot 0.02 \, \text{ml CO} \cdot \text{ml}^{-1} \, \text{Hb} \cdot \text{mg}^{-1} \, \text{Hb} = 16.7^* 22.7^+ 26.2^+ < 0.001
\]

\[
\text{COHbmeas}, \, \% = 0.79^* 3.17 4.09^+ < 0.001
\]

\[
\text{RO-HC}, \, \% \, \text{volume} = 43.7 20.7 26.1 < 0.006
\]

\[
\text{GGA}, \, \% \, \text{volume} = 9.73 0.06 0.23 < 0.006
\]

\[
\text{Lung density}, \, \text{g} / \text{ml} = 0.29 0.06 0.23 < 0.006
\]

\[
\text{NO} / (\text{d} \cdot \text{z}) = 4.76^* 23.8^+ 27.8^+ < 0.001
\]

\[
\text{VC,}\, \text{ml} = 50.1 \pm 1.67^* \quad 22.7 \pm 12.9^* \quad 33.5 \pm 19.5^+ < 0.001
\]

\[
\text{VC,}\, \text{TLCpleth} = 31.3 \pm 12.4^+ \quad 53.0 \pm 23.8^+ < 0.001
\]

\[
\text{VC,}\, \text{TLCpleth} = 2.87^* 13.5^* < 0.001
\]

\[
\text{VC,}\, \text{TLCpleth} = 0.80^* 4.38 \quad 0.79^* 3.17 < 0.001
\]

\[
\text{VC,}\, \text{TLCpleth} = 5.40^* 28.8 < 0.001
\]

\[
\text{VC,}\, \text{TLCpleth} = 1.645 \text{ corresponding to the 5th percentile of the } z \text{ score. This indicates how much a measurement differs from the predicted value, with a } z \text{-score of } -1.645 \text{ corresponding to the 5th percentile of the }
\]

Data are means ± SD. DLsCO and DLsCOdual dual lung diffusing capacity for NO and CO; TLCpleth, plethysmographically-determined TLC; DMCO, and VC, alveolar membrane conductance for CO (DMCO), and pulmonary capillary volume (VC) calculated by assuming an infinite (\( \theta_{\text{NO}} \)) value for NO specific blood conductance (\( \theta_{\text{NO}} \)), DMCO,7.66 and VC,7.66. DMCO and VC were calculated by assuming a finite value for \( \theta_{\text{NO}} \). Pair of symbols (*, †, ‡) indicates statistically significant pairwise differences between groups by Holm-Sidak or Dunn’s method. Other abbreviations are as given in Table 1.

Predicted values for DLsNO-DLsCOdual, DMCO, and VC, were from Agustini et al. (1).

**Chest CT scanning.** In all patients, within 30 days from pulmonary function testing, a thin-section CT of the entire chest was routinely obtained, at inspiratory breath-hold, in supine position by a multidetector row spiral scanner (SOMATOM Emotion 6, Siemens AG Medical, Forchheim, Germany). Image reconstruction was performed with a slice thickness of 1-mm increments. Density of the whole lung was calculated from CT number (Hounsfield units) and parenchymal density of the right hemidiaphragm, and their volumes computed by a tridimensional segmentation software (ITK-Snap 3.2, Philadelphia, PA) (Fig. 1). In each patient, the total percentages of lung volume with GGA and/or RO-HC were visually bounded (26) and their volumes computed by a tridimensional segmentation software. Other abbreviations are as given in Table 1.

Table 2. *Pulmonary diffusion data of healthy controls, UIP-IPF, and NSIP patients*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 81)</th>
<th>UIP-IPF (n = 30)</th>
<th>NSIP (n = 30)</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLsNO, ml·min⁻¹·mmHg⁻¹</td>
<td>109.3 ± 21.6†</td>
<td>35.3 ± 12.6‡</td>
<td>56.8 ± 21.8‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLsNO/VA, ml·min⁻¹·mmHg⁻¹</td>
<td>20.0 ± 12.2†</td>
<td>11.0 ± 2.8*</td>
<td>13.5 ± 3.5‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLsCOdual, ml·min⁻¹·mmHg⁻¹</td>
<td>23.0 ± 5.44*</td>
<td>8.62 ± 3.60*</td>
<td>13.4 ± 5.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLsCOdual/VA, ml·min⁻¹·mmHg⁻¹</td>
<td>4.19 ± 0.34*</td>
<td>2.61 ± 0.79*</td>
<td>3.17 ± 1.01†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLsNO/DLsCOdual</td>
<td>4.80 ± 0.24*</td>
<td>4.26 ± 0.80*</td>
<td>4.38 ± 0.80†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VA/TLCpleth</td>
<td>0.94 ± 0.05</td>
<td>0.92 ± 0.11</td>
<td>0.92 ± 0.08</td>
<td>0.24</td>
</tr>
<tr>
<td>DMCO,7.66, ml·min⁻¹·mmHg⁻¹</td>
<td>55.5 ± 10.9†</td>
<td>17.9 ± 6.39†</td>
<td>28.8 ± 11.1†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VC,7.66, ml</td>
<td>67.3 ± 22.5†</td>
<td>30.4 ± 17.3*</td>
<td>45.1 ± 26.2†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DMCO,7.66/VA, ml·min⁻¹·mmHg⁻¹</td>
<td>10.2 ± 0.60+</td>
<td>5.58 ± 1.46*</td>
<td>6.88 ± 1.81†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VC,7.66/Vz, ml·1⁻¹</td>
<td>12.1 ± 2.10+</td>
<td>9.32 ± 4.31*</td>
<td>10.7 ± 5.49†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DMCO,7.66, ml·min⁻¹·mmHg⁻¹</td>
<td>109.2 ± 16.8+</td>
<td>31.3 ± 12.4+</td>
<td>53.0 ± 23.8†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VC,7.66, ml</td>
<td>50.1 ± 16.7+</td>
<td>22.7 ± 12.9*</td>
<td>33.5 ± 19.5†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DMCO,7.66/VA, ml·min⁻¹·mmHg⁻¹</td>
<td>20.3 ± 2.13†</td>
<td>9.87 ± 3.67*</td>
<td>12.7 ± 4.76†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VC,7.66/VA, ml·1⁻¹</td>
<td>8.98 ± 1.56+</td>
<td>6.93 ± 3.20*</td>
<td>7.99 ± 4.09†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD. DLsCO and DLsCOdual dual lung diffusing capacity for NO and CO; TLCpleth, plethysmographically-determined TLC; DMCO, and VC, alveolar membrane conductance for CO (DMCO), and pulmonary capillary volume (VC) calculated by assuming an infinite (\( \theta_{\text{NO}} \)) value for NO specific blood conductance (\( \theta_{\text{NO}} \)), DMCO,7.66 and VC,7.66. DMCO and VC were calculated by assuming a finite value for \( \theta_{\text{NO}} \). Pair of symbols (*, †, ‡) indicates statistically significant pairwise differences between groups by Holm-Sidak or Dunn’s method. Other abbreviations are as given in Table 1.
There were no significant differences in arterial blood gases DLCO,stand were significantly higher in UIP-IPF (2.83 ± 0.54) patients. Both DMCO,stand and DLCO,dual were not significantly different between UIP-IPF and NSIP patients. The analysis of covariance (ANCOVA) was used to compare regression lines by testing the effect of the categorical factor UIP-IPF or NSIP on each dependent variable while controlling for the effect of a continuous covariate. To assess inter-rater agreement for CT scans reading, a Cohen’s weighted kappa coefficient (K) with 95% confidence interval (95% CI) was used (MedCalc Statistical Software, Version 15.8, Ostend, Belgium). Data are reported as means ± standard deviation (SD), with P < 0.05 being considered statistically significant.

RESULTS

The mMRC dyspnea score was significantly (P < 0.001) higher in UIP-IPF (2.83 ± 0.75) than NSIP (1.87 ± 1.04) patients.

Standard lung function. Spirometry, lung volumes, and DLCO,stand were significantly (P < 0.001) reduced in the whole IIPs group compared with matched controls (Table 1). Absolute values of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and total lung capacity (TLC) were significantly (P < 0.001) lower in UIP-IPF than NSIP patients. There were no significant differences in arterial blood gases although the partial pressure of oxygen in arterial blood (PaO2) tended to be less in UIP-IPF than NSIP (P = 0.07).

DLNO-DLCO,dual. DLNO absolute values were significantly decreased in IIPs compared with control subjects (46.0 ± 20.7 vs. 109.3 ± 21.6 ml·min⁻¹·mmHg⁻¹; P < 0.001) and in UIP-IPF significantly (P < 0.001) more than in NSIP patients (Table 2) but this difference disappeared when DLNO was divided by alveolar volume (DLNO/Vₐ). DLCO,dual values were also significantly less in IIPs than control group (11.0 ± 5.31 vs. 23.0 ± 5.44 ml·min⁻¹·mmHg⁻¹; P < 0.001) but did not differ significantly between UIP-IPF and NSIP (P > 0.05). DLNO/DLCO,dual ratio was significantly lower in IIPs than in control subjects (4.32 ± 0.79 vs. 4.80 ± 0.24; P < 0.001) but not significantly different (P > 0.05) in UIP-IPF vs. NSIP patients. Both DMCO,ν (23.4 ± 10.5 ml·min⁻¹·mmHg⁻¹) and DMCO,7.66 (42.1 ± 21.8 ml·min⁻¹·mmHg⁻¹) were severely decreased in IIPs compared with controls (55.5 ± 10.9 and 109.2 ± 16.8 ml·min⁻¹·mmHg⁻¹, respectively; P < 0.001 for both) and significantly (P < 0.001) lower in UIP-IPF than in NSIP. Both Vc,ν (37.8 ± 23.3 ml) and Vc,7.66 (28.1 ± 17.2 ml) were significantly decreased in IIPs compared with controls (67.3 ± 22.5 and 50.1 ± 16.7 ml, respectively; P < 0.001), but did not significantly differ between UIP-IPF and NSIP patients (P > 0.05). As for DLNO absolute values, correction for lung volume abolished differences between UIP-IPF and NSIP but not differences between patients and control subjects.

Chest CT scan. Total lung tissue density was significantly (P = 0.006) higher in UIP-IPF than in NSIP patients (Table 3). Because the between-observer agreement was good for both GGA (K = 0.77; 95% CI: 0.70–0.83) and RO-HC (K = 0.72; 95% CI: 0.63–0.80) % volume, the mean values were used for analysis. GGA % volume was significantly larger in NSIP than UIP-IPF (P = 0.006) whereas RO-HC % volume was significantly (P < 0.001) larger in UIP-IPF than NSIP.

Relationships between lung diffusing capacity and CT data. For the whole IIPs group, total lung density was weakly correlated with DLCO,stand (R² = 0.12; P = 0.007) and DLNO (R² = 0.11; P = 0.011) but insignificantly with DLCO,dual (R² = 0.06; P = 0.06).

For patients, both DLCO,stand and DLCO,dual were negatively correlated with RO-HC extent (R² = 0.51 and 0.48, respectively).

Fig. 2. Correlations between pulmonary diffusion measures and CT data in 30 UIP-IPF (open symbols) and 30 NSIP (closed symbols) patients. Top: standard lung diffusing capacity for carbon monoxide (DLCO,stand, diamonds). Middle and bottom: dual lung diffusing capacity for nitric oxide (DLNO, triangles) and carbon monoxide (DLCO,dual, circles). Data are expressed as z-score values and reticular opacity-honeycombing (RO-HC) % CT volume. Horizontal dashed lines indicate the 5th percentile of reference values (~1.645 z-score) and the shaded areas the range of values observed in healthy controls.

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DISCUSSION

The main findings of the present study are that in IIPs 1) DLNO was below the LLN in all patients irrespective of extent and nature of disease, and its decrement was more closely correlated with RO-HC amount than either DLCO,stand or DLCO,dual; 2) DMCO loss was also closely correlated with RO-HC extent and always below the LLN in both UIP-IPF and NSIP; and 3) VC was weakly correlated with RO-HC extent in UIP-IPF and uncorrelated in NSIP, with normal values in half of patients.

Comments on methodology. This study was carried out by DLNO-DLCO,dual measurement and subsequent derivation of DMCO and VC values (9, 22). This technique has been proposed as an alternative to solve the classical Roughton and Forster equation (44) with a single maneuver by simply adding NO to CO as a marker gas. In this analysis, θNO values have been assumed as either infinite (4, 22, 34) or finite (4, 7, 8, 34). Even though recent data in vivo (7, 8) pointed out that a value of 4.5 ml NO·ml blood⁻¹·min⁻¹·mmHg⁻¹ (10) may be considered as the most appropriate, the much higher overall affinity and, more importantly, faster reaction rate of NO than CO with free HbO2 (35) makes the assumption of a negligible erythrocyte resistance to NO justified for clinical purposes (24). This view is further supported by the present findings of DMCO,∞ and Vc,∞ being strictly and linearly correlated with DMCO,7.66 and Vc,7.66, respectively.

The present study has some methodological limitations. First, only 7 of 60 IIPs patients underwent a surgical lung
biopsy to confirm UIP-IPF or NSIP diagnosis. However, in our series of patients we used a multidisciplinary approach by integrating clinical, high-resolution CT scan and functional data as recommended by current international statement (45). Previous studies have shown that this approach has a good agreement with histopathological criteria for both UIP-IPF and NSIP pattern, which makes surgical lung biopsy not essential for diagnosis (16, 25, 41). Second, our method of visual lung segmentation of CT scan has not been validated against histopathologic scoring in IIPs. However, the interobserver reliability of our method was substantial and K-values very similar to those obtained in previous studies using a semiquantitative scale to assess the degree of ground glass attenuation and fibrotic changes (15, 26). A more complete evaluation of pathological changes in IIPs could be obtained by the analysis of CT-attenuation histograms over the whole lung but studies using this approach failed to show superiority to visual analysis in separating GGA and RO-HC (6, 12, 27). Because honeycombing is a mixture of high- and low-attenuation areas, the average density may not give correct information on RO-HC, which is the fundamental alteration of UIP-IPF (12, 49). Moreover, algorithm-based segmentation failed to include subpleural space, which is a major site of RO-HC in UIP-IPF (12). For these reasons, visual segmentation has been used as a reference for the validation of segmentation algorithm to separate GGA from fibrosis (6, 12, 27, 51). Third, we used breath-hold times of ~5 s for DLNO-DLCO,dual and 9–11 s for DLCO,stand measurements, respectively. The shorter breath-hold time of the dual method is the standard for the commercially available systems using electrochemical cell-based analyzers and has been used to generate the only available European predicting equations (1). However, the absolute values of DLCO,stand and DLCO,dual were strongly correlated between each other (r = 0.96; P < 0.001, data not shown). Fourth, recent data suggest that 40 ppm of NO inhaled could attenuate the hypoxic pulmonary vasoconstriction and potentially complicate the interpretation of pulmonary diffusion data (3). However, these effects were noted in healthy humans with local PaO2 < 60 mmHg (20), thus well below the range of 100 ± 4 mmHg calculated in our patients. In addition, Zavosky et al. (52) have shown that NO inhaled during sequential single-breath maneuvers has no effect on DLNO and the derived DMCO and VC values.

Comments on results. In a recent study, Wémeau-Stervinou et al. (50) did not show any correlation between DLCO components and either ground glass or interstitial scores in a lumped group of patients with UIP-IPF or NSIP. The present study also showed no correlation between ground glass extension and DLCO components but, at variance with the above report, strong correlations were found between DLNO and DMCO decrements and fibrotic changes extent. This discrepancy may be explained by methodological differences. First, the present study was not based on a subjective scoring system but used a volumetric assessment of fibrosis that was only in part observer-dependent. Second, the range of fibrosis extent in the present study was determined at six instead of three levels down the lung and the resulting range of fibrosis extent was much larger than in the previous study, thus more suitable for correlation analysis. Third, in the present series of patients DLCO and VC values were corrected for [Hb mea] and [COHb mea] rather than using [Hb stand]. Fourth, diffusion data in the present study were expressed as z-score values instead of absolute values, which are age-, sex-, and height-biased. Indeed, when absolute values were used in the present study the correlations between DMCO and RO-HC were substantially weaker than using z-scores.

Clinically relevant findings of the present study are that both DLCO,stand and DLCO,dual measurements were insensitive to the presence of fibrosis with an extent as large as 35% of lung parenchyma, whereas DLNO was below LLN in all patients and above it in all controls. Thus, using a z-score of −1.645 as a cut-off value, DLNO had 100% sensitivity and specificity for...
RO-HC whereas DL\textsubscript{CO,stand} and DL\textsubscript{CO,dual} had also specificity of 100% but sensitivity of 83% and 75%, respectively. This difference was apparently due to the variability of V\textsubscript{C}, which was within the range of normality in a nonnegligible number of patients. Because, CO is a gas mainly weighted by reaction limitation with blood H\textsubscript{b} rather than diffusion across alveolar membrane (9, 22, 34), a preserved or increased V\textsubscript{C} may mask the effect of fibrotic changes on DL\textsubscript{CO}. The reasons for variable V\textsubscript{C} values are a matter of speculation. One possibility could be that capillary blood flow is diverted away from lung regions with hypoxic vasoconstriction to normoxic regions (2) or from obliterated vessels to nonfibrotic regions (28). Thus the net effect on total V\textsubscript{C} would be determined by the relative contribution and prevalence of these compensatory mechanisms of vascular anatomic derangements.

A more specific physiological assessment of alveolar membrane diffusion can be provided by using NO, the transport of which is virtually independent of reaction with H\textsubscript{b} (7, 8). DL\textsubscript{NO} and DM\textsubscript{CO} decrements were greater in UIP-IPF than NSIP patients, suggesting a greater increment of barrier thickness or a greater loss of alveolar surface area due to more severe fibrotic changes in the former (21). The fact that DL\textsubscript{NO}/VA values were not differently decreased in UIP-IPF than NSIP patients, suggesting that the major difference between these two diseases was a greater reduction in lung volume, as also shown by the different values of TLC. According to current guidelines on UIP-IPF (40), a reduction of DL\textsubscript{CO} values below 40% of predicted is considered as a poor prognostic index, more than FVC (29, 31) or other lung function parameters including TLC, P(A-a)O\textsubscript{2} and maximum O\textsubscript{2} uptake (11). In the present series, 37 of 60 patients showed DL\textsubscript{NO} but not DL\textsubscript{CO,stand} values < 40% of predicted and 30 had a DL\textsubscript{NO} but not DL\textsubscript{CO,stand} z-score < -3 (Fig. 7).

The results of the present study suggest that ground glass regions have no significant effects on DL\textsubscript{NO} and DL\textsubscript{CO}. The lack of correlation between pulmonary diffusion data and GGA might reflect a compensatory increase in blood flow to nonfibrotic inflamed or edematous regions (2, 28).

Conclusions. The results of the present study show that DL\textsubscript{NO} measurement may provide a more sensitive evaluation of fibrotic changes than DL\textsubscript{CO,stand} in either UIP-IPF or NSIP. An advantage of DL\textsubscript{NO} is that it resulted in a value below the LLN when the extent of fibrosis was as small as ~10% of lung volume, which was not always the case for DL\textsubscript{CO}. The present findings may represent a rationale for longitudinal studies to evaluate the usefulness of DL\textsubscript{NO} for prognosis and follow-up in IIPs patients.

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This study had trial registry (https://clinicaltrials.gov) PRS: No. NCT02596841 (IRCCS 2015).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
AUTHOR CONTRIBUTIONS

Author contributions: G.B. conception and design of research; G.B. performed experiments; G.B., C.B., and A.G. analyzed data; G.B. and V.B. interpreted results of experiments; G.B. prepared figures; G.B. and V.B. drafted manuscript; G.B., M.B., and V.B. edited and revised manuscript; G.B. and V.B. approved final version of manuscript.

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Fig. 6. Correlations between volume-corrected diffusion components [DMCO,∞/VA (A and B) and VC,∞/VA (C and D)] and CT data in UIP-IPF and NSIP. The shaded areas show the range of values observed in healthy controls. Symbols and other abbreviations are as given in Fig. 5.

Fig. 7. Relationship between DLCO,stand (diamonds) or DLNO (triangles), expressed as z-score (horizontal axis) and % of predicted (vertical axis) values, in 30 UIP-IPF (open symbols) and 30 NSIP (closed symbols) patients. Horizontal and vertical dashed lines indicate 40% of predicted and −3 z-score values, respectively.
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