Source of Exhaled Nitric Oxide in Primary Biliary Cirrhosis*

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**Background:** Exhaled nitric oxide (NO) levels may be elevated in patients with liver cirrhosis and autoimmune diseases. Primary biliary cirrhosis (PBC) is often associated with keratoconjunctivitis sicca (Sjögren syndrome [SS]), an extrahepatic autoimmune manifestation. The aim of this study was to evaluate the source of increased exhaled NO (ie, alveolar vs airway) in patients with PBC, whether associated with SS or not, and to evaluate its impact on oxygenation abnormalities.

**Design:** Observational controlled study.

**Setting:** University hospital.

**Methods:** The fractional alveolar NO concentration (FANO) and airway flux of NO (QbrNO) were measured by the multiple flows technique in 34 patients with PBC, 12 with associated SS, and were compared to 20 control subjects and 12 patients with primary SS.

**Results:** FANO was significantly higher in patients with PBC, associated with SS (mean [± SEM], 8.9 ± 0.8 parts per billion [ppb]) or not (mean, 7.7 ± 0.7 ppb) compared to healthy control subjects (mean, 4.6 ± 0.5 ppb; p < 0.001) and to patients with primary SS (mean, 4.3 ± 0.5 ppb; p < 0.001). FANO was significantly higher in cirrhotic patients with increased alveolar-arterial oxygen pressure difference (P[A-a]O₂) compared to patients with normal P[A-a]O₂ values (9.8 ± 0.8 vs 7.3 ± 0.7, respectively; p = 0.018). When compared with control subjects and with patients with PBC not associated with SS, QbrNO was significantly increased in patients with both primary SS and SS associated with PBC.

**Conclusions:** Increased exhaled NO levels found in PBC are from both alveolar and airway sources in patients with associated SS, but only FANO is associated with oxygenation impairment.

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**Key words:** exhaled nitric oxide; hepatopulmonary syndrome; liver cirrhosis; Sjögren syndrome

**Abbreviations:** CA = alveolar concentration; CE = contrast-enhanced; DLCO = diffusing capacity of the lung for carbon monoxide; FENO = fractional exhaled nitric oxide concentration; HPS = hepatopulmonary syndrome; NO = nitric oxide; P[A-a]O₂ = alveolar-arterial oxygen pressure difference; PBC = primary biliary cirrhosis; ppb = parts per billion; QbrNO = airway flux of nitric oxide; SS = Sjögren syndrome; Vexh = flow rate; VNO = nitric oxide output

Nitric oxide (NO) has been suggested to be an important mediator of excessive intrapulmonary vasodilatation, which is one of the mechanisms causing oxygenation impairment in patients with liver cirrhosis. A positive relationship between exhaled NO and the alveolar-arterial oxygen pressure difference (P[A-a]O₂) has been reported in patients with liver cirrhosis, and high levels of exhaled NO are commonly observed in patients with hepatopulmonary syndrome (HPS), which is defined by the presence of hypoxemia due to intrapulmonary vasodilatation or arteriovenous shunts in patients with liver disease. According to experimental data, the increased NO production in patients with liver cirrhosis should depend on the overactivity of constitutive NO synthase in endothelial cells of the lung vasculature, possibly due to the stimulation of endothelin-1 B-type receptors by increased circulating levels of endothelin-1. An increased number of macrophages...
sequestered in pulmonary microvessels, and the increased expression and activity of inducible NO synthase also have been shown in a murine model of HPS.9 High levels of the fractional exhaled NO concentration (FENO) are also commonly observed in patients with asthma, in whom FENO is considered to be a marker of airway inflammation. The site of production of NO may help to understand the different pathophysiologic consequences of an increased production of NO by respiratory system. By using multiple flow rate (Vexh) measurements of FENO, it is possible to calculate NO output from alveolar and bronchial origins.9 By using this technique, Deleaux et al.10 were able to differentiate the increase in FENO from the alveolar source, which they had observed in patients with liver cirrhosis, from the increase in FENO from the bronchial source, which they had observed in patients with asthma.

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that is characterized by the progressive destruction of biliary epithelial cells and is caused by immunologic factors.11 Coexistent extrahepatic autoimmune disease states arise in many patients, and keratoconjunctivitis sicca (ie, Sjögren syndrome [SS]) is the most frequent disorder, occurring in about 30 to 75% of patients.12 Elevated levels of exhaled NO have been reported in patients with primary SS.13 Airway epithelial cells, macrophages, and lymphocytes infiltrating the airways may be the source of increased NO production.13,14 The aim of the study was to evaluate whether patients with PBC and associated SS had more elevated levels of respiratory NO output than patients with PBC without SS. We also wanted to investigate the source of exhaled NO in patients with PBC, both associated with and not associated with SS. Our hypothesis was that the source of increased exhaled NO levels in patients with PBC would be from the alveoli, whereas in patients with PBC and SS the source would be from both the alveoli and the conducting airways. We expected also to find that increased alveolar NO concentrations could negatively influence oxygenation in patients with PBC, according to one study.10 To this aim, we analyzed the alveolar and conducting airway components of exhaled NO in a group of patients with PBC, with or without SS, and compared them to those of healthy subjects and of patients with primary SS.

**Materials and Methods**

**Subjects**

**Patients With PBC:** Thirty-four patients with PBC who were attending an outpatient clinic for PBC (Ospedale Gradeno; Torino, Italy) were referred for pulmonary function tests. All patients were nonsmokers, and none had received a diagnosis of asthma or chronic obstructive lung disease. All patients had antimitochondrial antibodies in their serum and biopsy-proven diagnoses of PBC. The severity of PBC was characterized by the Mayo risk score,15 which was derived from a Cox regression model that used the following variables: serum bilirubin and serum albumin levels; age; prothrombin time; and clinical severity of edema. Twelve patients also had received a diagnosis of secondary SS, based on the European criteria.16 For the diagnosis of SS, four of the following six criteria are necessary: (1) subjective complaints of xerophthalmia (specific questionnaire); (2) subjective complaints of xerostomia (specific questionnaire); (3) ocular signs of keratoconjunctivitis; (4) histopathologic features in the labial minor salivary gland biopsy that are compatible with focal sialoadenitis; (5) evidence of salivary gland involvement (specific tests); and (6) the presence of autoantibodies to extractable nuclear and cytoplasmic antigens, Ro/SS-A or La/SS-B, in the sera.

**Patients With Primary SS:** Twelve nonsmoking patients with primary SS who were being followed up as outpatients were studied. The onset of disease was 2 to 10 years prior to entering the study (mean, 6 years). None of the SS patients was receiving inhalation therapy.

**Healthy Control Subjects:** Twenty subjects (15 female) were nonsmokers, were receiving no medication, and had no evidence of cardiopulmonary or allergic disease at the time of the study. The subjects had normal spirometry findings.

**Exhaled NO Measurement**

The online measurement of the FENO was performed according to American Thoracic Society recommendations,17 using a chemiluminescent NO analyzer (model 280 NOA; Sievers; Boulder, CO). After a full inspiration of room air through an acid gas filter (NO, < 5 parts per billion [ppb]) [AFL 1410; Sievers], subjects exhaled against a positive pressure of 11 to 16 cm H2O to generate Vexh values of 50, 100, 150, and 200 mL/s. For each Vexh, the NO output (VNO = Vexh × FENO) was calculated.

FENO is inversely related to Vexh, whereas VNO varies directly as a function of Vexh. At Vexh values of > 50 mL/s, the latter relationship is linear and can be expressed as VNO = CA × Vexh + JNOair,max,18,19 where CA is the alveolar concentration and JNOair,max is maximal airway output. The positive slope of this relationship (ie, CA) represents the steady-state CA of NO in parts per billion, and the y intercept (ie, JNOair,max) approximates the maximum total flux of NO (in nanoliters per second) from the airway wall into the lumen that would occur at infinite Vexh.

**Pulmonary Function**

Spirometry, flow-volume curves, residual volume, and diffusing capacity of the lung for carbon monoxide (DLco) [single-breath measurement] were performed (Vmax 22 system; Sensor-Medics; Yorba Linda, CA) according to American Thoracic Society guidelines.19,21 Predicted values were taken from Quanjer22 and from Cotes.23

**Arterial Blood Gas Analysis**

Arterial blood gas samples were obtained by percutaneous radial artery puncture with the subject in a seated upright position and were analyzed (model 330; ABL; Copenhagen, Denmark). P(A–a)O2 was calculated using the alveolar gas equation, and it was considered abnormal if > 20 mm Hg.
**Contrast-Enhanced Echocardiography**

All the patients with P(A-a)O₂ values of > 20 mm Hg were investigated for the presence of intrapulmonary vascular dilatations by saline solution contrast-enhanced (CE) transthoracic echocardiography, which was performed by use of a peripheral IV line, as previously reported. A CE echocardiographic study that was indicative of intrapulmonary vascular dilatation was defined as having a delayed appearance (ie, three to six beats after the initial appearance of contrast in the right side of the heart) of microbubbles in the left side of the heart. Patients with P(A-a)O₂ values of > 20 mm Hg and positive CE echocardiographic study findings were considered to have HPS.

**Statistical Analysis**

The data are presented as the mean ± SE. Analysis of variance with the Bonferroni correction of multiple comparisons was used to evaluate the differences between groups. Pearson correlation coefficients were used to assess the association between exhaled NO measures, and clinical and pulmonary function test parameters, with adjustments for multiple comparisons. Statistical significance was defined as p < 0.05.

**RESULTS**

The demographic and clinical features of study subjects are reported in Table 1. No significant differences were observed in age, pulmonary function test results, P(A-a)O₂, and Mayo risk score between cirrhotic patients with and without SS. Patients with PBC had significantly lower DLCO compared to patients with primary SS, who had normal values on pulmonary function test results. Twelve of 22 patients with PBC and 3 of 12 patients with PBC associated with SS had P(A-a)O₂ values of > 20 mm Hg (p = 0.15). Three patients with PBC, all without SS, had the criteria for the diagnosis of HPS (ie, positive CE echocardiography findings with increased P[A-a]O₂ values of 31, 24, and 25 mm Hg).

**Exhaled NO**

Table 2 summarizes the exhaled NO measurements. FENO values at an expiratory flow rate of 50 mL/s were significantly higher in patients with PBC, associated or not with SS, and in patients with primary SS than in control subjects. In patients with both primary SS and SS associated with PBC, the resultant y-intercept of the VNO vs Vexh plots was significantly increased, indicating increased conducting airway flux of NO (QbrNO) into the airway lumen (Fig 1). On the other hand, patients with PBC that was not associated with SS had normal QbrNO. FENO at the highest flow (ie, an expiratory flow rate of 200 mL/s) and the slope of the VNO vs Vexh plot were significantly increased in all patients with PBC, indicating a higher alveolar NO concentration compared with those of control subjects and patients with primary SS.

**Exhaled NO and Lung Function**

Cirrhotic patients with increased (ie, > 20 mm Hg) mean P(A-a)O₂ values (slope, 9.8 ± 0.8 ppb) had higher alveolar NO concentrations compared to patients with normal P(A-a)O₂ values (slope, 7.3 ± 0.7 ppb; p = 0.018) [Fig 2]. The three patients who had the criteria for the diagnosis of HPS had among the highest alveolar NO concentrations (slope, 12, 11, and 13 ppb). There was no relation between exhaled NO measurements and DLCO in patients with PBC.

**Exhaled NO and Severity of PBC**

No correlation was found between any exhaled NO measurements and Mayo risk scores.

**DISCUSSION**

The main findings of the study were that alveolar NO concentrations are increased in patients with PBC, and that in those patients with PBC and associated SS, as well as in those with primary SS, the maximum QbrNO is also increased compared with that of control subjects.

**Table 1—Demographic and Clinical Features of Study Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>PBC/SS− (n = 22)</th>
<th>PBC/SS+ (n = 12)</th>
<th>SS (n = 12)</th>
<th>Control Subjects (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59.2 (2.2)</td>
<td>56.4 (2.1)</td>
<td>52.3 (4.2)</td>
<td>49.2 (6.5)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>12</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>105 (3.7)</td>
<td>104 (5)</td>
<td>99 (5)</td>
<td>104 (3)</td>
</tr>
<tr>
<td>FEV₁/VC ratio</td>
<td>77 (1.2)</td>
<td>78 (1)</td>
<td>76 (1.5)</td>
<td>79 (1.2)</td>
</tr>
<tr>
<td>TLC, % predicted</td>
<td>96.5 (3.9)</td>
<td>103 (5.2)</td>
<td>99 (4.3)</td>
<td></td>
</tr>
<tr>
<td>DLCO, % predicted</td>
<td>80 (4.3)†</td>
<td>86 (3.3)‡</td>
<td>92 (3.5)</td>
<td></td>
</tr>
<tr>
<td>P(A-a)O₂, mm Hg</td>
<td>23.9 (7.9)</td>
<td>17.8 (9.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayo risk score</td>
<td>3.9 (0.1)</td>
<td>4.1 (0.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values given as mean (SEM). SS− = without SS; SS+ = with SS; TLC = total lung capacity. †p < 0.05 compared to SS.
By using the multiple Vexh technique, it is possible to differentiate whether the increase in exhaled NO was from an alveolar or bronchial source. The technique is based on the observation that the measurement of FENO depends on the rate of expiratory flow. FENO is inversely related to Vexh, whereas NO output varies directly as a function of ventilation, according to a linear relationship at a Vexh of >50 mL/s. NO concentration is stable at the alveolar level and is increased by bronchial excretion during the passage of exhaled air through the airway. The clinical and pathophysiologic value of partitioning the source of exhaled NO is underlined by the observation that elevated FENO in asthma patients has been clearly demonstrated to originate from the conducting airways and has been correlated with airway hyperresponsiveness.

Increased NO output in exhaled air previously has been reported in patients with liver cirrhosis and Delclaux et al were able to demonstrate that increased NO output in patients with liver cirrhosis is of alveolar origin and is correlated with P(A-a)O₂. High values of exhaled NO actually have been reported in patients with HPS, in whom oxygenation impairment is due to excessive intrapulmonary vasodilation, which is thought to be in part mediated by NO. Even if in the present series of patients with cirrhosis we could not find a significant correlation between alveolar NO concentration and P(A-a)O₂, it is noteworthy that patients with increased P(A-a)O₂ had a mean alveolar NO concentration that was significantly higher than that of patients with normal P(A-a)O₂.

### Table 2—Summary of Exhaled NO Measurements*

<table>
<thead>
<tr>
<th>Variable</th>
<th>PBC/SS− (n = 22)</th>
<th>PBC/SS+ (n = 12)</th>
<th>SS (n = 12)</th>
<th>Control Subjects (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FENO₅₀ ppb</td>
<td>21.5 (1.2)†</td>
<td>27.1 (3.1)†</td>
<td>22.4 (2.4)†</td>
<td>14.1 (1.1)</td>
</tr>
<tr>
<td>FENO₂₀₀ ppb</td>
<td>12 (1.1)¶</td>
<td>12.1 (1.1)¶</td>
<td>8.1 (0.9)</td>
<td>7.5 (0.8)</td>
</tr>
<tr>
<td>y-intercept, NO nL/s</td>
<td>0.57 (0.1)</td>
<td>1.1 (0.1)¶</td>
<td>1.05 (0.2)¶</td>
<td>0.55 (0.1)</td>
</tr>
<tr>
<td>Slope, ppb</td>
<td>8.9 (0.8)¶</td>
<td>7.7 (0.7)¶</td>
<td>4.3 (0.9)</td>
<td>4.6 (0.5)</td>
</tr>
</tbody>
</table>

*Values given as mean (SEM). FENO₅₀ = FENO at expiratory flow rate of 50 mL/s; FENO₂₀₀ = FENO at expiratory flow rate of 200 mL/s. See Table 1 for abbreviations not used in the text.

†p < 0.05 compared to control subjects.
‡p < 0.001 compared to control subjects.
¶p < 0.05 compared to PBC/SS−.
||p < 0.001 compared to SS.

**Figure 1.** Plots of the mean NO output vs expiratory Vexh relationship in patients with PBC, associated with SS (●) and not associated with SS (▲), in patients with primary SS (■) and in control subjects (□). The slope depends on CA (significantly higher in PBC compared to control subjects and primary SS), while the y-intercept depends on the maximal QbrNO (significantly higher in patients with both primary SS and SS associated with PBC).

**Figure 2.** CA of NO (CANO) in patients with PBC with increased and normal P(A-a)O₂ (AaO₂) values.
and that the three patients who had the criteria for the diagnosis of HPS had elevated NO production from an alveolar source. The lack of correlation between exhaled NO and oxygenation impairment in the present series of patients with cirrhosis could be explained by considering that they were outpatients who had less severe oxygenation impairment compared with patients who are attending a liver transplant unit, where the prevalence of HPS is far more common. According to experimental data, the increased alveolar NO concentration in patients with liver cirrhosis could be due to increased NO production, which is dependent on the overactivity of both the constitutive isoform of NO synthase and the inducible isoform.

Another theoretical explanation for the increased alveolar NO concentrations could be related to a decreased diffusing capacity of NO from the alveolar space into the pulmonary capillary blood. This mechanism has been suggested to explain the high alveolar NO concentration found in patients with systemic sclerosis, in whom Girgis et al found an inverse correlation between alveolar NO concentration and DLCO. A decreased diffusion of NO from the alveolar region seems unlikely in patients with liver cirrhosis, in whom pulmonary capillaries are larger than normal. Our patients actually had a decreased diffusion capacity for CO, as would be expected in patients with liver cirrhosis. Nevertheless, we could not find a significant correlation between the decrease in DLCO and the increase in alveolar NO concentration in our patients.

The other finding of our study was that SS is associated with increased maximum QbrNO. Increased NO in the exhaled air of patients with primary SS previously has been reported. We have shown here that the increased NO output in patients with primary SS is of airway origin, and that in patients with PBC and SS the increased NO output is both of an airway and alveolar origin, but it is only the FENO coming from an alveolar source that is related to oxygenation abnormalities in patients with PBC. In patients with primary SS, it has been suggested that elevated levels of NO, which is activated by cytokines released from lymphocytes or by inflammatory cells infiltrating the airway mucosa, may derive from the airway epithelium.

In conclusion, by using multiple Vexh measurements of exhaled NO, we were able to demonstrate that increased NO levels found in PBC patients are from both alveolar origins, in which excessive NO concentration may contribute to the oxygenation impairment observed in HPS patients, and from an airway origin in patients with associated SS.

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REFERENCES
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