Nasal Nitric Oxide Measurements To Screen Children for Primary Ciliary Dyskinesia*

Regula Corbelli, MD; Bettina Bringolf-Isler, MD; Arnold Amacher, MD; Bernd Sasse, MD; Max Spycher, MD; and Jürg Hammer, MD

Study objective: To examine the usefulness of exhaled and nasal nitric oxide (NO) measurements to detect primary ciliary dyskinesia (PCD) in children.

Design and methods: The study population consisted of 34 children with symptoms suggestive of PCD who were previously referred to our pediatric university respiratory disease clinic for a diagnostic workup including analysis of ciliary structure and function by respiratory mucosal biopsy. PCD was diagnosed in 17 of the 34 children according to the ciliary biopsy results. Measurements of nasal and exhaled NO were performed according to European Respiratory Society and American Thoracic Society guidelines in the patients with and without biopsy-proven PCD, and also in 24 healthy age-matched subjects.

Results: Nasal NO was significantly lower in those children with proven PCD (geometric mean; 13.7 parts per billion [ppb]), compared to those who had negative biopsy results (132.7 ppb) and healthy control subjects (223.7 ppb). The measurement of nasal NO in our study population showed, below a cut-off level of < 105 ppb, a specificity of 88% for PCD, and positive predictive value of 89%. Nasal NO above a cut-off level of 105 ppb excluded PCD with a 100% certainty. The lower levels of exhaled NO in patients with PCD did not reach statistical significance.

Conclusion: The measurement of nasal NO appears to be a useful tool to screen children for PCD and to exclude this disease in those with high nasal NO levels.

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Key words: bronchiectasis; bronchitis; children; exhaled nitric oxide; Kartagener syndrome; nasal nitric oxide; primary ciliary dyskinesia; situs inversus

Abbreviations: NO = nitric oxide; PCD = primary ciliary dyskinesia; ppb = parts per billion

Primary ciliary dyskinesia (PCD) is a recessively inherited group of disorders of ciliary structure and/or function resulting in impaired mucociliary clearance. The clinical manifestations are recurrent or chronic respiratory tract infections with mucus retention leading to sinusitis, serous otitis media, rhinitis, and bronchitis. Situs inversus occurs in 50% of the children, and male infertility is common. Progression of lung disease is variable and affected by time of diagnosis, ability of medical treatment to control the symptoms, and prevention of complications such as the development of bronchiectasis. The incidence of PCD in the white population is estimated to be 1 in 15,000. In children with recurrent respiratory diseases, PCD can be found in approximately 5.6%.1,2

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cause secondary ciliary dyskinesia following epithelial injury from viral respiratory tract infections or exposure to pollutants is not always easy to exclude. Hence, it is not surprising that the diagnosis of PCD is often missed for a long time.

The presence of nitric oxide (NO) in exhaled breath of humans was first demonstrated by Gustafsson et al in 1991. It was shown later that large amounts of NO are constantly released in the nasal airways and the paranasal sinuses. It has been suggested that the measurement of nasal NO concentrations may serve as a diagnostic tool, because children with PCD have very low levels of exhaled and nasal NO compared to healthy children or children with cystic fibrosis, bronchiectasis, or asthma. The aim of this retrospective study was to investigate whether the measurement of nasal NO would reliably identify PCD in a group of children who were previously referred to our institution to exclude explicitly this diagnosis by respiratory mucosal biopsy, because of chronic or recurrent respiratory tract infections or situs inversus.

**MATERIALS AND METHODS**

**Subjects**

We recruited a group of 34 children (mean age, 11.4 ± 1.2 years [± SE]) who were referred to our pulmonary center at the University Children’s Hospital Basel during the period of 1994 to 2002, because of clinical symptoms suggestive of PCD. These children had recurrent or chronic upper and lower respiratory tract infections (n = 25) or newly diagnosed situs inversus (n = 9). Cystic fibrosis was excluded by routine sweat testing in all children except those with known situs inversus. All children underwent respiratory mucosal biopsy sampling to confirm or exclude PCD through analysis of ciliary motility and ciliary ultrastructure by light and electron microscopy. A clear-cut abnormality of ciliary ultrastructure was necessary to establish the diagnosis of PCD. All respiratory mucosal biopsy samples were obtained from the nose or from a mainstem bronchus and analyzed at a university pathology laboratory specialized in the diagnosis of PCD by electron microscopy. Ultrastructural defects consistent with a diagnosis of PCD were confirmed in 13 children and excluded in 14 children (labeled as PCD-negative patients). Diagnostic details are summarized in Table 1. A defined ultrastructural defect occurring in the majority of the cilia was required for the diagnosis of PCD. Most PCD-negative patients had secondary impaired ciliary motility at the time of biopsy, reflecting the presence of recurrent or chronic respiratory tract infections at the time of referral. The occurrence of compound cilia was regarded as secondary alterations.

Twenty-four healthy children (mean age, 12.4 ± 1.0 years) volunteered to serve as a control group for this study. At the time of measurement, all children were clinically stable and none had any clinical evidence of an acute respiratory tract infection, such as fever, increased sputum production, shortness of breath, or cough. No patients were receiving antibiotics at the time of NO measurements.

**Table 1—Diagnostic Details of Study Population**

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrastructural defects of children with PCD (n=17)</td>
<td></td>
</tr>
<tr>
<td>Reduced number of inner dynein arms</td>
<td>2</td>
</tr>
<tr>
<td>Absence of inner dynein arms</td>
<td>9</td>
</tr>
<tr>
<td>Reduced number of outer dynein arms</td>
<td>1</td>
</tr>
<tr>
<td>Tubular defects</td>
<td>5</td>
</tr>
<tr>
<td>Clinical diagnosis of children without PCD (n=17)</td>
<td></td>
</tr>
<tr>
<td>Non-PCD bronchiectasis</td>
<td>1</td>
</tr>
<tr>
<td>Postinfectious bronchiolitis obliterans</td>
<td>1</td>
</tr>
<tr>
<td>Plastic bronchiitis</td>
<td>1</td>
</tr>
<tr>
<td>Recurrent or chronic bronchiectasis</td>
<td>14</td>
</tr>
</tbody>
</table>

**NO Measurement**

NO was measured using a chemiluminescence analyser (Exhalizer D; Eco Medics; Dürnten, Switzerland) with a measurement range from 0.1 to 5,000 parts per billion (ppb) [detection limit of 0.06 ppb, rise time < 100 ms, and sampling rate of 10 ms]. In addition to NO, flow was measured by an ultrasound spirometer (flow range, ± 16 L/s; accuracy, ± 2%; dead space, 7.2 mL) and CO2 by mainstream capnography (accuracy, ± 2 mm Hg; response time, < 120 ms). All tests were performed with ambient NO levels < 50 ppb, usually < 10 ppb. No children were receiving steroids or antibiotics at the time of the NO measurements.

**Nasal Sampling**

Nasal NO was measured in the children sitting with an olive inserted inside one nostril ensuring a tight seal while the contralateral nostril was left open. Air was continuously sampled at a constant transnasal flow rate of 20 mL/s with the soft palate closed. The constant transnasal flow produced a washout phase of NO followed by the establishment of a steady plateau documented in the profile of NO (Fig 1). The transnasal air flow was measured by an ultrasonic spirometer. A breath hold for 10 s or a slow exhalation against a resistance was performed to achieve soft palate closure. Nasal CO2 concentration was measured to verify velum closure and exclude contamination by alveolar gas. Three consecutive measurements were performed, and the values were averaged. The method of NO measurements was based on the 1999 American Thoracic Society guidelines.

**Exhaled NO Measurement**

Exhaled NO from the lower airways was measured after inhalation of NO-free air according to the American Thoracic Society/European Respiratory Society recommendations. Hence, patients able to cooperate performed a slow vital capacity maneuver by exhaling against a fixed resistance at a flow rate of 0.05 L/s.

**Ethics**

Informed consent was obtained from all individuals and/or their parents. The measurement of exhaled NO and the study was approved by our local ethics committee.

**Data Analysis**

Data are presented as geometric means and 95% confidence intervals (CIs). For comparison between means, a one-way analysis of variance was used to determine a significant difference
between the levels of NO and disease. An unpaired t test was used in a bivariable way to compare the different groups; p values < 0.05 were considered to indicate statistical significance. The receiver operated curve, sensitivity, and specificity of NO levels for the diagnosis of PCD were calculated.

RESULTS

All patients and control subjects completed the study for the nasal NO measurements. Unfortunately, exhaled NO measurements of eight children (four in each patient group) had to be excluded because of technical problems or inability to perform a correct maneuver. Individual data of nasal NO levels are displayed in Figure 2 and of exhaled NO levels in Figure 3. Means of nasal and exhaled NO measured in 17 children with PCD (age, 12 ± 2 years) were 13.7 ppb (95% CI, 6.8 to 27.8 ppb) and 2.9 (95% CI, 1.9 to 4.6 ppb), respectively. Means of nasal and exhaled NO measured in the 17 PCD-negative patients (age, 10.5 ± 1.8 years) were 132.7 ppb (95% CI, 76.5 to 230.2 ppb) and 8.7 ppb (95% CI, 4.9 to 15.2 ppb), respectively. Means of nasal and exhaled NO measured in 24 healthy control subjects were 223.7 ppb (95% CI, 175.5 to 285.2 ppb) and 6.4 ppb (95% CI, 4.8 to 8.6 ppb), respectively. Nasal NO concentrations were significantly lower in children with PCD (p < 0.05). Although there was an obvious tendency toward lower exhaled NO levels in patients with PCD, the differences between the three groups did not reach statistical significance (p = 0.11), due to the smaller number of measurements and the smaller change in magnitude in exhaled vs nasal NO measurements.

If a nasal NO level of < 105 ppb is taken as diagnostic for PCD, this has a positive predictive value of 0.89 and a negative predictive value of 0.94. A nasal NO level < 105 ppb will have a sensitivity of 94% and a specificity of 88% for PCD. A level of nasal NO > 105 ppb excludes PCD with a 100% certainty in our study population of children. There were two patients who had nasal NO levels < 100 ppb but normal ciliary ultrastructure. One of these patients has recurrent plastic bronchitis and sinusitis, and had a nasal NO level of 80 ppb. The other child is of Tamil origin and had chronic sinusitis and nonallergic asthma. This patient had a repeatable nasal NO level of only 3 ppb. Ciliary motility was highly abnormal in both children. Both children had normal sweat test results, and were negative on skin-prick testing for all common allergens.

DISCUSSION

The results of our study demonstrate that nasal NO measurements may serve as a diagnostic tool to rule out PCD in children with chronic or recurrent respiratory symptoms. We found that nasal NO levels > 105 ppb excluded PCD with a 100% certainty in our patients with respiratory symptoms suggestive for this disease. Our study also indicates
that in the case of low nasal NO measurements PCD must be actively confirmed by additional means, because low nasal NO levels can also be found in other respiratory disorders such as cystic fibrosis, bronchiectasis, chronic sinusitis, and panbronchiolitis.6,12,13

This study assessed the diagnostic value of nasal and exhaled NO in a small group of children in whom PCD was strongly suspected and who were referred for more invasive and specific investigations for this disease. The only limitation of our study was that these patients were recruited retrospectively for NO measurements after PCD had been excluded or confirmed by respiratory mucosal biopsy. Only two other studies have previously measured nasal NO concentrations in children with PCD and compared them to children with other defined respiratory disorders such as PCD, bronchiectasis, cystic fibrosis, or asthma. Both studies demonstrated low levels of nasal NO in patients with an established diagnosis of PCD. Narang et al6 reported that nasal NO levels < 250 ppb showed a sensitivity of 97% and a specificity of 90% for the diagnosis of PCD. Horvath et al9 found that nasal NO readings < 187 ppb identified PCD patients from other bronchiectatic patients, with a specificity of 98% and a positive predictive value of 92%. We showed that nasal NO levels < 105 ppb identified PCD in patients with symptoms suggestive for this disease, with a specificity of 94% and a positive predictive value of 89%. The difference in the cut-off levels is explained by the difference in nasal airflow during sampling of 20 mL/s in our study and 4 mL/s in the other two studies. This highlights the importance of standardizing the technique and defining individual laboratory reference values as long as custom-made equipment is used.14 Although reduced levels of exhaled NO are also characteristic for PCD, our study confirms previous findings6,7 that measurements of exhaled NO do not discriminate patients with PCD from healthy subjects as clearly as measurements of nasal NO.

The possibility to exclude PCD by a simple measurement of nasal NO concentrations seems clinically very attractive, because the diagnosis of PCD is a difficult one and involves the complex assessment of ciliary structure and function. Electron microscopy is an essential part of diagnostic testing to ensure that secondary ciliary dysfunction from acute or chronic infection is not confused with PCD. Ultrastructural studies have to demonstrate a clear-cut abnormality of ciliary structure, but in rare cases this is not always possible. In addition, primary ciliary dyskinesia has been described without ultrastructural abnormalities, sometimes with primary ciliary disorientation.15,16 There is only one other test that has been used to screen patients for PCD, the saccharin test. This test is almost impossible to perform properly in children because of its duration and need for

![Figure 2. Individual measurements of nasal NO concentrations in patients with symptoms suggestive of PCD and healthy control subjects. PCD was confirmed (PCD+) or excluded (PCD-) by electronmicroscopy of respiratory mucosal biopsy samples. Median and 25th and 75th percentiles are also displayed for each group.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22017/)

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subjective interpretation. Nasal NO levels > 105 ppb excluded PCD with a 100% certainty in our study population, and would have avoided more invasive and costly investigations for these patients. The availability of an easy and noninvasive screening test for PCD is also attractive, because this may facilitate early diagnosis. The measurement of nasal NO may also be valuable in patients with inconclusive biopsy results. It must, however, be considered that low levels of nasal NO may also occur in children with cystic fibrosis, non-PCD bronchiectasis, or sinus disease of any etiology due to obstruction of sinus ostia reducing transfer of NO from the paranasal sinuses. We found low levels of nasal NO in two children without PCD, one with plastic bronchitis due to severe asthma, and the other believed to have chronic sinusitis and nonallergic asthma with incomplete response to bronchodilators. Although the reason for the low nasal NO concentrations is unknown, it is recognized that nasal NO correlates with impaired mucociliary function, and that reduced NO synthesis may contribute to the chronic airway infections present in both our children. Nakano et al13 described low levels of nasal NO in patients with panbronchiolitis and hypothesized that whatever the pathogenesis of a disease, chronic sinusitis per se can potentially decrease nasal NO concentrations because of the widespread epithelial damage in paranasal sinuses resulting from recurrent inflammation.13 In fairness, we are unable to exclude that these two children are affected by a rare form of PCD characterized by abnormal ciliary orientation and absence of ultrastructural defects.

The reason for the low exhaled and nasal NO concentrations in patients with PCD has not been fully clarified. Several observations suggest that NO plays an important role in signal transduction associated with ciliary functions. The epithelial NO synthase is ultrastructurally localized to the basal body of the microtubules of the cilia, and NO has been found to stimulate ciliary beat frequency.17–19 It seems unlikely, however, that the lower levels of nasal and exhaled NO in PCD are the results of reduced NO synthase activity, because levels on NO metabolites are not different between patients with PCD and healthy subjects.20

In conclusion, we showed that measurements of nasal NO levels are helpful to screen children with clinical symptoms suggestive for PCD and to decide on the need for further, more invasive testing. If nasal NO is unexpectedly low in a patient with recurrent respiratory infections, the diagnosis of PCD should be actively excluded while a high nasal NO points against the diagnosis of PCD. A prospective study in a larger patient population including adults is required to establish the technology further as a valuable diagnostic tool in respiratory medicine.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22017/)

**Figure 3.** Individual measurements of exhaled NO concentrations in patients with symptoms suggestive of PCD and healthy control subjects. PCD was confirmed (PCD+) or excluded (PCD−) by electronmicroscopy of respiratory mucosal biopsy samples. Median and 25th and 75th percentiles are also displayed for each group.
REFERENCES