Nasal Nitric Oxide Levels and Nasal Polyposis in Children and Adolescents With Cystic Fibrosis

Karin M. de Winter–de Groot, MD; Sanne van Haren Noman, MD; Lucienne Speleman, MD; Anna G. M. Schilder, MD, PhD; Cornelis K. van der Ent, MD, PhD

IMPORTANCE The counterintuitive decrease of exhaled nitric oxide (NO) levels in a severe inflammatory disorder like cystic fibrosis (CF) is only scarcely understood. Because NO is important in a variety of regulatory processes in the lung, including host defense, inflammation, and bronchomotor control, it is necessary to search for clarifying mechanisms.

OBJECTIVES To explore whether fractional exhaled NO (FENO) and nasal NO (nNO) levels are associated with CF genotype, nutritional status, presence of nasal polyps, pulmonary function, and airway colonization with Staphylococcus aureus and Pseudomonas aeruginosa in children with CF, and to investigate the effect of functional endoscopic sinus surgery (FESS) on FENO and nNO levels in children with CF and persistent sinonasal disease.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study (association with NO) and prospective study (effect of FESS on NO) in a tertiary care referral center. Patients included 95 children with CF in clinically stable condition at routine annual multidisciplinary examination, 13 of whom were referred for a FESS procedure.

INTERVENTIONS Functional endoscopic sinus surgery in children with CF and persistent sinonasal disease.

MAIN OUTCOMES AND MEASURES Body mass index (BMI), FENO and nNO levels, results of flexible nasal endoscopy, pulmonary function tests (forced expiratory volume in 1 second and forced vital capacity), and airway cultures.

RESULTS Children with nasal polyposis have significantly lower nNO levels than those without polyposis (median, 53 vs 140 parts per billion; \( P = .001 \)); these values are negatively associated with colonization with \( S \) aureus (β = −.22; \( P = .04 \)). After FESS, nNO values increase significantly, although not to normal levels.

CONCLUSIONS AND RELEVANCE In children with CF, the presence of nasal polyps is associated with significantly lower nNO levels than in children without nasal polyps. After FESS for nasal polyposis, nNO levels increase significantly, but not to normal levels. Low nNO levels are associated with \( S \) aureus colonization in the oropharynx and lower airways.

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Nitrergic oxide (NO) is a free radical gas and messenger molecule that is produced by respiratory epithelial cells and is important in a variety of regulatory processes in the lung, including host defense, inflammation, and bronchomotor control. High levels of NO are detectable in the nasal cavity and paranasal sinuses. The NO concentrations measured in exhaled air are generally increased in chronic inflammatory lung diseases, such as asthma and bronchiectasis.

Although cystic fibrosis (CF) is a severe inflammatory disease of the airways, studies show that fractional exhaled NO (F<sub>e</sub>NO) and nasal NO (nNO) levels are not increased in this disorder but are normal or even reduced. Almost all patients with CF have lower airway disease with airflow obstruction, 90% have chronic sinusitis, and 25% to 50% have nasal polyposis.

There are several possible explanations for these counterintuitive reduced NO values in patients with CF. The formation of NO could be decreased owing to a lack of the substrate L-arginine in patients with a poor nutritional status. Moreover, thick mucus lining in the airways and obstruction of nasal sinuses by nasal polyps might prevent diffusion of NO into the gaseous phase or might increase NO metabolism, and CF genotype and NO synthase polymorphisms might be related to NO levels in patients with CF.

Studies on the determinants of NO in inflammatory diseases are scarce, and the dynamics of NO in this disease are only partially understood.

Because the decrease in NO levels in a severe inflammatory disorder such as CF is scarcely understood, we performed a cross-sectional study to determine whether CF genotype, nutritional status, presence of nasal polyps, pulmonary function, and bacterial colonization are associated with F<sub>e</sub>NO and nNO levels in children with CF. We also prospectively investigated the effect of functional endoscopic sinus surgery (FESS) on F<sub>e</sub>NO and nNO levels in children with CF and persistent symptoms of sinonasal polyposis.

### Methods

#### Patient Characteristics

We studied 95 clinically stable children and adolescents (median age, 12.6 years; range, 5.3-18.6 years) with CF during their routine annual multidisciplinary examination at the Cystic Fibrosis Center Utrecht. The study protocol was approved by the Medical Ethical Committee of the University Medical Center, Utrecht, the Netherlands.

The CF mutations were classified in 2 ways: classic homozygous ΔF508 mutation vs compound heterozygous mutation and “severe” (class I-III) vs “mild” (class IV and V) mutation. Patients with severe mutations had pancreatic insufficiency.

The body mass index (BMI) was expressed as the z score (SD) for BMI. Routine ear, nose, and throat examination was performed in all patients by an experienced pediatric otolaryngologist (A.G.M.S.). Nasal endoscopy was performed with a 2.2-mm flexible endoscope.

### Pulmonary Function Tests

Pulmonary function tests were performed (Masterlab; Viasys Healthcare), including forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC), before and after administration of 800 μg of salbutamol sulfate. Reversibility was calculated by subtracting FEV<sub>1</sub> before salbutamol administration from FEV<sub>1</sub> after salbutamol administration. The FEV<sub>1</sub> and FVC values were expressed as percentages of predicted values.

Sputum cultures or oropharyngeal cough swab samples were collected routinely. Chronic infection (ie, colonization) with *Pseudomonas aeruginosa* and *Staphylococcus aureus* was defined as at least 2 consecutive cultures that were positive for *P aeruginosa* and *S aureus* during the last 6 months before the annual examination.

### Measurements of NO

Nitric oxide levels were measured with a chemiluminescence analyzer (NIOX; Aerocrine AB) designed to perform measurements according to the guidelines of the American Thoracic Society and European Respiratory Society. The analyzer was regularly calibrated according to the manufacturer’s instructions with standard gas mixtures of NO (211 and 2060 parts per billion [ppb] for bronchial and nasal measurements, respectively) and certified NO-free gas (HoekLoos). To exclude the effect of ambient NO levels on the nNO levels, all tests were performed with ambient NO concentrations below 10 ppb. No nitrate-rich consumptions were used before the NO measurements.

The F<sub>e</sub>NO level was measured during a single-breath exhalation for 10 seconds against a mouth pressure of 10 cm H<sub>2</sub>O at a flow rate of 50 mL/s. These values are reported as means of 3 repeated measurements.

To measure nNO, an ergonomically designed tightly fitting nasal sampling olive was inserted into the patient’s right nostril, completely occluding the nostril to avoid ambient air sampling. The contralateral nostril was left open for breathing. The nNO levels were measured by using a humming method at 128 Hz. After a deep inhalation of room air, the children were asked to hum at a frequency of 128 Hz during nasal exhalation with the aid of a tuning fork. Air was sampled from the right nostril with a flow rate of 5 mL/s. Values were measured using the last 4 seconds of the humming exhalation, when a stable end-expiratory plateau was reached. Because it has been reported that nNO during humming decreases during repeated maneuvers, the children were asked to repeat the humming exhalations 6 times. The last 3 cycles were used to calculate a mean nNO concentration.

The NO measurements were performed before other pulmonary function tests.

In a second phase, we also studied 13 children with CF before and after FESS. Children were selected for FESS if they had persistent symptoms of nasal obstruction, rhinorrhea, postnasal drip, headache, or hyposmia; some patients also had a decline in lung function due to extensive sinonasal polyposis. The FESS procedure was performed by an experienced pediatric otolaryngologist (A.G.M.S. or L.S.).

In all children, we measured F<sub>e</sub>NO and nNO levels and pulmonary function just before surgery and 3 to 6 months after.
At the same time, we noted BMI and use of nasal corticosteroids and we obtained sputum cultures or oropharyngeal cough swab samples to assess colonization with P aeruginosa and/or S aureus.

Statistical Analysis
To assess NO levels, we calculated the mean of triplicate measurements. Analyses were performed on log-transformed values of FENO and nNO because these values were not normally distributed. We used t tests to compare NO levels between groups and performed univariate analyses to analyze the relationship between NO and all determinants. When the results of univariate analysis revealed a possible association (P < .10), variables were included in a multivariate analysis. For the multivariate analysis, multiple linear regression models were used. Paired t tests were used to study differences in NO levels, pulmonary function, BMI, and colonization status before and after FESS. Differences were considered statistically significant at P < .05. All analyses were done with SPSS software (version 12.0 for Windows; SPSS Inc).

Results
Characteristics of the study populations are given in Table 1 and Table 2. All measurements were performed in all patients. The cystic fibrosis transmembrane regulator genotype was not available in 13 patients. The median FENO level for all subjects was 9.8 ppb (range, 1.7-59.6 ppb), and the median nNO level was 110 ppb (range, 5-792 ppb). The distribution of nNO values is shown in Figure 1. No correlation was found between FENO or nNO and age or sex. Thirty-nine patients used intranasal corticosteroids (fluticasone propionate). In this group, 23 (59%) of the patients had nasal polyps and 16 (41%) did not. Not all patients with nasal polyps used intranasal corticosteroids, and the mean nNO levels did not differ between patients who used intranasal corticosteroids and those who did not (P = .60). No patients used systemic corticosteroids.
Genotype and Nutritional Status
At univariate and multivariate analysis, the cystic fibrosis transmembrane regulator genotype and BMI (z score) showed no significant correlation with FENO or nNO (Table 3 and Table 4).

Nasal Polyps
One-third of the children with CF (31 of 95) had sinonasal polyps, which was negatively associated with nNO (P = .007; Table 4). These patients had significantly lower nNO levels than those without polyps (median, 53 vs 140 ppb; P = .001). The FENO levels in patients with sinonasal polyps did not differ from those in patients without polyps (median, 10.9 vs 9.8 ppb; P = .80).

Pulmonary Function and Colonization Status
There was no significant correlation between nNO or FENO levels and pulmonary function. Of the 19 patients who did not receive FESS, 12 (63%) were chronically colonized with S aureus compared with 11 (85%) in the FESS group (P < .05). Of the 31 patients with nasal polyps, 23 (74%) were colonized with S aureus compared with 27 of 64 patients (42%) who did not have nasal polyps (P < .05).

In the multivariate analysis, a significant correlation was found between nNO levels and colonization with S aureus. Patients with chronic P aeruginosa infection had significantly lower FENO levels in the univariate analysis, but this correlation was not significant in the multivariate analysis (Tables 3 and 4).

Before and After FESS
After FESS in children with CF and nasal polyps, nNO levels increased significantly, although not to normal levels (Figure 2).

The FENO, pulmonary function test results, and BMI values did not differ before and after surgery. All children used nasal corticosteroids before and after FESS. After FESS, fewer sputum cultures were positive for S aureus than before the operation (4 vs 11 positive cultures, respectively; P = .03). No differences were found between preoperative and postoperative P aeruginosa colonization.

Discussion
Sinonasal polyps and S aureus colonization are associated with low nNO levels in children with CF, which increase after surgery to levels comparable to those in patients with CF without polyps. These data shed new light on the dynamics of NO in CF. The paranasal sinuses are an important source of NO production, and mechanical obstruction might contribute to re-

Table 3. Univariate and Multivariate Analysis of Determinants for FENO Levels in 95 Patients With Cystic Fibrosis

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<tr>
<th>Determinant</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
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<tr>
<td></td>
<td>β Value</td>
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<td>Staphylococcus aureus colonization</td>
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<td>Pseudomonas aeruginosa colonization</td>
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</tr>
<tr>
<td>FEV1</td>
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<td>.06</td>
</tr>
<tr>
<td>Reversibilitya</td>
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<td>FVC</td>
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<td>.08</td>
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Abbreviations: BMI, body mass index; FENO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity.

* Tested as response in FEV1 to administration of 800 μg salbutamol per inhalation.

Table 4. Univariate and Multivariate Analysis of Determinants for nNO in 95 Patients With Cystic Fibrosis

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Univariate Analysis</th>
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<tr>
<td>FVC</td>
<td>−.003</td>
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</tr>
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</table>

Abbreviations: BMI, body mass index; FENO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; nNO, nasal nitric oxide.

* Tested as response in FEV1 to administration of 800 μg salbutamol per inhalation.
In the paranasal sinuses, where very high concentrations of NO can be measured, NO seems to represent the first line of host defense, contributing to sterility of these cavities.\(^2\)\(^4\)

In the present study, children with CF and low nNO values were more frequently colonized by \textit{S. aureus} compared with \textit{P. aeruginosa} in the lower respiratory tract. The lack of association with \textit{P. aeruginosa} colonization might be age related; studies that have found associations between low exhaled NO concentrations and \textit{P. aeruginosa} colonization have mainly been conducted in adolescents or adults with CF. In several non-CF studies, a role in killing \textit{S. aureus} was assigned to NO and nitrogen intermediates.\(^25\)\(^-\)\(^26\) In patients with Wegener granulomatosis (WG), \textit{S. aureus} colonization has been associated with an increased risk of a relapse involving the upper respiratory tract. Haubitz et al\(^27\) reported nNO levels, but normal F\(_2\)NO levels, in patients with active vs inactive Wegener granulomatosis. This suggests that low nNO levels may compromise host defense in the upper airways, thus contributing to infection and colonization with \textit{S. aureus} and further promoting disease activity. Our finding of higher nNO levels and fewer \textit{S. aureus}-positive cultures after FESS support this explanation.

We found no association between \textit{F}_2\text{NO} or nNO levels and genotype, lung function, BMI, or pancreatic status. The lack of correlation with lung function is in line with findings of some others\(^1\)\(^1\)\(^,\)\(^2\)\(^1\)\(^,\)\(^2\)\(^8\)\(^-\)\(^2\)\(^9\) but in contrast with those reported by Keenan et al\(^1\); they found that patients with pancreatic insufficiency and severe mutations had lower NO levels than pancreatic-sufficient patients with mild mutations, although the comparison groups were small, as were the reported differences in \textit{F}_2\text{NO} and nNO levels. Similar to our results, other authors have not found any correlation between NO levels and genotype.\(^2\)\(^1\)\(^-\)\(^2\)\(^0\)

The presence of NO synthase polymorphisms may be related to patients’ NO levels. Grasemann et al\(^1\)\(^2\) concluded from their data that variants in the NOS1 gene are associated with decreased formation of NO in the upper airways of patients with CF. Zhang et al\(^3\)\(^1\) found some single-nucleotide polymorphisms for NOS1 that were associated with chronic rhinosinusitis, but these were different from those found by Grasemann et al. These findings may be promising but need to be confirmed. Large studies are required to find convincing evidence for an association between gene polymorphisms and a parameter (eg, NO levels) or a disease (eg, nasal polyposis).

In conclusion, in children with CF, those with sinonasal polyps have significantly lower nNO levels than those without polyps. After FESS for persistent sinonasal polyposis, nNO levels in these patients increase significantly, not to normal levels but to levels comparable to those in patients with CF without polyps. Furthermore, low nNO levels are associated with \textit{S. aureus} colonization in the oropharynx and lower airways.
Critical revision of the manuscript for important intellectual content: Speelman, Schilder, van der Ent.

Statistical analysis: de Winter-de Groot, van Haren Noman, van der Ent.

Study supervision: Speelman, Schilder, van der Ent.

Conflict of Interest Disclosures: None reported.

REFERENCES