Relationship between Exhaled Nitric Oxide and Small Airway Lung Function in Normal and Asthmatic Children

Naoki Nakajima1, Hiroyuki Mochizuki2, Reiko Muramatsu1, Satomi Hagiwara1, Takahisa Mizuno1 and Hirokazu Arakawa1

ABSTRACT

Background: In children, exhaled nitric oxide (eNO) is usually confounded by factors such as age and height. We evaluated the relationship between eNO and lung function by minimizing the effects of aging and height.

Methods: In Study 1, the subjects comprised 738 elementary school children and junior high school children (aged 6 to 15 years, 366 males and 372 females). They were divided into two groups according to age (6-10 years and 11-15 years). A height range was determined by a histogram of height in each group. In Study 2, lung function, respiratory resistance and eNO level were measured in age- and height-limited groups.

Results: In Study 1, total of 148 younger children ranging in height from 120 to 130 cm and 180 older children ranging in height from 148 to 158 cm participated in Study 2. The level of eNO among asthmatic children was higher than that of normal children in both the younger and the older groups. The decrease in forced expiratory volume in 1 second (FEV1) and other parameters of central airway resistance did not correlate with the eNO level. However, the small airway parameters of MMEF and V25/HT in older asthmatic children, and V25/HT and R5-R20 in younger asthmatic children inversely correlated with eNO.

Conclusions: Our data suggest that eNO level inversely correlates with small airway narrowing, and airway inflammation has a significant effect on small airway lung function in asthmatic school children.

KEY WORDS

asthma, children, exhaled nitric oxide, lung function, respiratory resistance

INTRODUCTION

The measurement of exhaled nitric oxide (eNO) has been proposed as a non-invasive marker of airway inflammation in asthmatic patients.1-4 The eNO is useful for diagnosing childhood asthma as well as for predicting asthma relapse in children with clinical asthma remission.5-7 Recent studies have demonstrated that eNO levels are decreased by anti-inflammatory treatment,8 and the measurement of eNO has been proposed as a method of monitoring the efficacy of treatment in asthmatic patients.9

However, previous research concerning the effect of acute airway narrowing on eNO level, reported a rapid reduction in the eNO level after methacholine challenge,10,11 and this reduction did not correlate with the decrease in forced expiratory volume in 1 second (FEV1). In addition, several authors reported that eNO values increase following β2-agonist therapy.12,13 Despite the large number of studies of eNO in children, the relationship between lung functions and eNO levels has remained unclear because measurements are often confounded by factors such as age and height, which have a potent effect on eNO level.

In the present study, we sought to evaluate whether the eNO level in normal and asthmatic children correlates with lung function and respiratory resistance in large and small airway passages measured by the impulse oscillometry system. Because it is well known that age and height have a remarkable effect on eNO levels in children,14-16 we strictly limited
these factors in the study participants.

METHODS

SUBJECTS

In 2007 and 2008, we performed a health check using questionnaires and lung function tests in 738 children from 2 elementary schools and one junior high school in Maebashi City, Gunma Prefecture, Japan. To control for the effect of age and height, the children were categorized according to height ranges determined by a histogram of height.

To diagnose asthma, the parents of each child completed an ATS/DLD respiratory questionnaire in Japanese. If the child had experienced more than 3 episodes of wheezing and was diagnosed asthma by a physician, the child was determined to be asthmatic. All of the asthmatic children were well controlled, and participated in the school curriculum. Furthermore, none of the study subjects regularly used inhaled steroids, inhaled long-acting beta agonists and oral steroids, but an asthmatic subject in the older group used pranlukast, leukotriene antagonist. Approval was obtained from the Gunma University Hospital Ethics Committee, and informed parental consent for each child was obtained before the study.

STUDY DESIGN

In Study 1, the parents of students in 2 elementary schools and one junior high school were asked to complete a questionnaire regarding their child's history of wheezing and respiratory diseases. At 15:00-18:00 from September to November in 2007 and 2008, lung function, airway resistance and eNO were measured at each school. The eNO level was measured prior to the evaluation of lung function and respiratory resistance.

The height and weight of each subject were measured by school teachers within one week before the study. The height range categories for Study 2 were determined by a histogram of height using data from Study 1.

OFF-LINE eNO COLLECTION AND ANALYSIS

The off-line eNO measurements were performed using a standardized device (Sievers Instruments, Boulder, CO, USA) as well as a modified device (Fig. 1).17 In order to monitor the oral pressure and the flow volume, we modified the standardized device and attached a flowmeter with a manometer. A plastic tube (internal diameter 3 mm and length 23 cm) was connected to a valve and worked as a flow restrictor. Using this modified device, the subjects exhaled through resistance, and maintained a constant pressure of 20-22 cmH₂O, which led to a constant flow of 50 ml/s. Using the standardized device, the subjects exhaled against a constant pressure of 20-22 cmH₂O, which led to a constant flow of 100 ml/s. All participants inspired NO-free medical air and performed one single exhalation into a NO-inert and impermeable 1.5-L Mylar balloon. Because the on-line eNO reaches a plateau 5 seconds after the beginning of exhalation in both adults and children,17 we instructed the subjects to continue exhaling for 10 seconds. After 5 seconds of exhalation, the operator attached the Mylar balloon to the plastic tube and collected the sample for the last 5 seconds. The sample was measur-
Table 1 Profiles of Patients

<table>
<thead>
<tr>
<th>Study 1</th>
<th>No.</th>
<th>m : f</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>eNO (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10 years</td>
<td>246</td>
<td>126 : 120</td>
<td>8.5 ± 1.1</td>
<td>124.9 ± 8.2</td>
<td>27.6 ± 7.2</td>
<td>30.2 ± 18.4</td>
</tr>
<tr>
<td>11-15 years</td>
<td>492</td>
<td>240 : 252</td>
<td>12.7 ± 1.3</td>
<td>152.6 ± 10.0</td>
<td>45.6 ± 11.5</td>
<td>34.8 ± 28.3</td>
</tr>
</tbody>
</table>

| Study 2 | 120-130 cm | Total | 148 | 70 : 78 | 8.5 ± 0.9 | 125.6 ± 3.3 | 24.7 ± 1.6 | 30.9 ± 18.3 |
| Normal | 114 | 53 : 61 | 8.5 ± 0.9 | 125.6 ± 3.3 | 24.6 ± 1.7 | 27.1 ± 14.3 |
| Asthma | 34 | 17 : 17 | 8.4 ± 0.9 | 125.9 ± 3.2 | 27.4 ± 4.5 | 43.4 ± 24.1* |

| 148-158 cm | Total | 180 | 119 : 61 | 12.9 ± 1.2 | 154.2 ± 3.0 | 48.2 ± 8.5 | 31.9 ± 27.1 |
| Normal | 133 | 85 : 48 | 13.1 ± 1.2 | 154.5 ± 2.5 | 48.7 ± 8.2 | 28.4 ± 20.0 |
| Asthma | 47 | 29 : 18 | 13.0 ± 1.1 | 151.8 ± 3.2 | 44.9 ± 8.5 | 40.5 ± 36.2* |

* p < 0.01 compared with normal subjects.

Table 2 Results of Lung Function Tests

<table>
<thead>
<tr>
<th>Study 1</th>
<th>FVC</th>
<th>FEV₁</th>
<th>MMEF</th>
<th>V₅₀</th>
<th>V₂₅-HT</th>
<th>R₅</th>
<th>R₅-R₂₀</th>
<th>X₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10 years</td>
<td>1.74 ± 0.4</td>
<td>1.56 ± 0.3</td>
<td>1.97 ± 0.5</td>
<td>2.23 ± 0.6</td>
<td>0.85 ± 0.3</td>
<td>0.71 ± 0.2</td>
<td>0.18 ± 0.1</td>
<td>-0.25 ± 0.1</td>
</tr>
<tr>
<td>11-15 years</td>
<td>2.83 ± 0.8</td>
<td>2.53 ± 0.7</td>
<td>3.03 ± 1.0</td>
<td>3.40 ± 1.2</td>
<td>1.14 ± 0.4</td>
<td>0.45 ± 0.1</td>
<td>0.08 ± 0.1</td>
<td>-0.19 ± 0.1</td>
</tr>
</tbody>
</table>

| Study 2 | 120-130 cm | Total | 1.69 ± 0.3 | 1.51 ± 0.2 | 1.88 ± 0.4 | 2.13 ± 0.5 | 0.81 ± 0.2 | 0.74 ± 0.2 | 0.18 ± 0.1 | -0.25 ± 0.1 |
| Normal | 1.69 ± 0.3 | 1.52 ± 0.2 | 1.90 ± 0.4 | 2.14 ± 0.5 | 0.83 ± 0.2 | 0.73 ± 0.2 | 0.18 ± 0.1 | -0.25 ± 0.1 |
| Asthma | 1.69 ± 0.2 | 1.49 ± 0.2 | 1.80 ± 0.5 | 2.08 ± 0.5 | 0.75 ± 0.2 | 0.74 ± 0.2 | 0.19 ± 0.1 | -0.25 ± 0.1 |

| 148-158 cm | Total | 2.92 ± 0.5 | 2.58 ± 0.4 | 3.03 ± 0.7 | 3.34 ± 0.8 | 1.17 ± 0.4 | 0.41 ± 0.1 | 0.05 ± 0.1 | -0.13 ± 0.1 |
| Normal | 2.93 ± 0.5 | 2.60 ± 0.4 | 3.14 ± 0.8 | 3.45 ± 0.9 | 1.22 ± 0.4 | 0.40 ± 0.1 | 0.05 ± 0.1 | -0.13 ± 0.1 |
| Asthma | 2.77 ± 0.4 | 2.41 ± 0.3* | 2.70 ± 0.7** | 2.99 ± 0.8** | 1.01 ± 0.3** | 0.43 ± 0.1 | 0.07 ± 0.1* | -0.14 ± 0.1 |

*p < 0.05, **p < 0.01 compared with normal subjects.

Pulmonary Function Tests

Pulmonary function was determined by spirometry with a calibrated computerized pneumotachograph spirometer (Jaeger Masterscope PC; Hoechberg, Germany; LAB Manager Software version 4.53.2, 2002). Standard measures of pulmonary function were collected, including FVC, FEV₁, and V₅₀, V₂₅-HT. The procedure for all pulmonary function tests involved the following pattern; (1) three normal tidal volume breaths, (2) inhalation to total lung capacity, (3) forced maximal exhalation, and (4) a final maximal inhalation. All tests were performed according to the current American Thoracic Society spirometry standards. The resting baseline was selected using the best-of-three resting results based on the highest sum of FVC and FEV₁. One maneuver was performed at each post-challenge time point in an effort to minimize subject fatigue and guard against the potential effects of deep inhalation. However, if any baseline or post-challenge time point measurement was technically unacceptable, the maneuver was repeated immediately.

Impulse Oscillography System

Airway reactivity in response to a methacholine inhalation challenge was determined by the impulse oscillometry system (Jaeger MS-IOS; Hoechberg, Germany; LAB Manager Software version 4.53.2, 2002) using the recommended techniques of the manufacturer. Real-time recordings of mouth pressure and flow signals pulsed through 5- to 20-Hz spectrum were superimposed over tracings of tidal breathing and displayed on a computer screen. Measurements of resistance at 5 and 20 Hz (R₅, R₂₀, R₅-R₂₀) and reactivity at 5 Hz (X) were recorded. It was agreed that...
the value of $R_{20}$ primarily demonstrates central respiratory resistance, and the value of $R_{5}-R_{20}$ demonstrates small respiratory resistance.\textsuperscript{11,18}

DATA ANALYSIS
Statistical analyses and data presentation were performed with the SPSS computer software package (version 11.0; SPSS, Chicago, IL, USA). The comparison of each parameter among normal and asthma subjects was performed with the Student's t-test. Correlations among lung functions and eNO levels were made by using the Pearson correlation test. Partial correlation was used to evaluate the effect of gender on the correlation between lung functions and eNO levels. Data are expressed as the mean $\pm$ SD. $p$ values $<0.05$ were considered to be significant.

RESULTS

STUDY 1
By a histogram of height, the mean $\pm$ SD of height was 124.5 $\pm$ 7.0 cm in the 6-10 year-old group, and 152.6 $\pm$ 11.0 cm in the 11-15 year-old group. As in our previous report, we determined a 10 cm height range (120-130 cm in the 6-10 year-old group, and 148-158 cm in the 11-15 year-old group) as the criteria for selecting subjects for further study.\textsuperscript{19}

A total of 148 younger children (120-130 cm, 8.5 $\pm$ 0.9 years) and 180 older children (148-158 cm, 12.9 $\pm$ 1.2 years) participated in Study 2 (Fig. 1). The numbers of normal children and asthmatic children were 114 and 34 in the younger group, respectively, and 133 and 47 in the older group, respectively (Table 1).

There were no significant differences in age, height or weight between the normal and asthmatic children.

STUDY 2
Results of eNO Measurements and Lung Function Tests
The eNO levels and lung function data are shown in Table 1, 2. In both the 120-130 cm group and the 148-158 cm group, the mean eNO level of asthmatic children was significantly higher than that of the normal subjects ($p < 0.001$, $p < 0.001$, Table 1).

In the 120-130 cm group, there was no difference in the results of the lung function tests and IOS between the asthmatic and normal subjects. However, in the 148-158 cm group, there were significant differences between the asthmatic and normal subjects in regard to the small airway parameters of $FEV_{1,MMER}$, $V_{50}$, $V_{25}/HT$ and $R_{5}-R_{20}$.

Relationship between Lung Function Parameters and eNO Level
In the 120-130 cm group, the eNO level in the total subjects did not correlate with FVC or $FEV_{1}$, but significantly correlated with $V_{25}/HT$ (Table 3a). When the children were grouped according to diagnosis, this tendency was evident in the asthmatic subjects but not in the normal subjects.

In the 148-158 cm group, the eNO level in the total subjects did not correlate with FVC and $FEV_{1}$, but significantly correlated with MMEF and $V_{25}/HT$ (Table 3b). This tendency was evident in the asthmatic

Table 3a  Correlation Coefficients and $p$ Values of eNO in Younger Children

<table>
<thead>
<tr>
<th>Height Range</th>
<th>FVC</th>
<th>FEV$_1$</th>
<th>MMEF</th>
<th>$V_{50}$</th>
<th>$V_{25}/HT$</th>
<th>$R_{5}$</th>
<th>$R_{5}-R_{20}$</th>
<th>$X_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>120-130 cm</td>
<td>CC</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>(n = 148)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.027</td>
<td>-0.031</td>
<td>-0.149</td>
<td>-0.104</td>
<td>-0.209</td>
<td>-0.121</td>
<td>-0.113</td>
<td>0.047</td>
</tr>
<tr>
<td>Health</td>
<td>-0.031</td>
<td>-0.043</td>
<td>-0.065</td>
<td>-0.050</td>
<td>-0.067</td>
<td>-0.033</td>
<td>-0.008</td>
<td>-0.095</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.171</td>
<td>0.034</td>
<td>-0.238</td>
<td>-0.174</td>
<td>-0.373</td>
<td>-0.342</td>
<td>-0.468</td>
<td>0.291</td>
</tr>
</tbody>
</table>

Table 3b  Correlation Coefficients and $p$ Values of eNO in Older Children

<table>
<thead>
<tr>
<th>Height Range</th>
<th>FVC</th>
<th>FEV$_1$</th>
<th>MMEF</th>
<th>$V_{50}$</th>
<th>$V_{25}/HT$</th>
<th>$R_{5}$</th>
<th>$R_{5}-R_{20}$</th>
<th>$X_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>148-158 cm</td>
<td>CC</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>(n = 180)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.086</td>
<td>-0.062</td>
<td>-0.163</td>
<td>-0.140</td>
<td>-0.225</td>
<td>0.040</td>
<td>0.077</td>
<td>-0.044</td>
</tr>
<tr>
<td>Health</td>
<td>0.054</td>
<td>0.050</td>
<td>0.018</td>
<td>0.036</td>
<td>-0.095</td>
<td>0.002</td>
<td>0.054</td>
<td>0.118</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.372</td>
<td>-0.127</td>
<td>-0.408</td>
<td>-0.303</td>
<td>-0.413</td>
<td>0.067</td>
<td>0.017</td>
<td>-0.177</td>
</tr>
</tbody>
</table>

\textsuperscript{11,18}
subjects but not in the normal subjects.

Relationship between IOS Parameters and eNO
In the 120-130 cm group, the eNO level in the total subjects did not correlate with R5, R5-R20 or X5 (Table 3a). However, in the asthmatic children, small airway respiratory resistance (R5-R20) was significantly correlated with eNO (Table 3a).

In the 148-158 cm group, the eNO level in the total subjects did not correlate with R5, R5-R20 or X5 (Table 3b). Furthermore, no correlation was observed in when the subjects were evaluated according to the asthma diagnosis.

The Effect of Gender and Asthma on the Relationship between Lung Function Parameters, IOS Parameters and eNO
The partial correlation coefficient was calculated by controlling for gender (Table 4). In the 120-130 cm group, the eNO level in the total subjects significantly correlated with V25/HT. In the 148-158 cm group, the eNO level in the total subjects significantly correlated with MMEF, V50 and V25/HT (Table 4).

DISCUSSION
Exhaled NO is a good parameter for evaluating airway inflammation in asthma patients. A number of previous studies have indicated that airway inflammation in asthma induces an increase in the eNO level,5,8 moreover, the eNO level demonstrates an inverse correlation with FEV1 and other parameters of lung function.20,21 However, de Gouw et al. observed a decrease in the eNO level after bronchial provocation with histamine and adenosine-5’-monophosphate, and this decrease significantly correlated with a decrease in FEV1.22 The authors concluded that the fall in the eNO level was not caused by the procedure itself but was related to an acute decrease in FEV1, thus suggesting that eNO levels are modulated by the degree of airway obstruction. Furthermore, some studies have reported that the eNO level is reduced after methacholine bronchial provocation.11-13

These reports have indicated that rapid airway narrowing results in a transient decrease in the eNO level. However, it has not been precisely determined whether chronic airway inflammation induces an increase in the eNO level in normal and asthmatic children, because many factors, particularly age and height, have a marked effect on childhood eNO level.14-16

We evaluated the relationships between the eNO level, lung function and respiratory resistance in normal and asthmatic children with strictly limited age and height. According to a previous study, the limitation of age and height can minimize the confounding effects of these factors.19 In addition, the limitation of age can minimize the risk of infection and environmental stimulation.

Our results demonstrated that the eNO level in asthmatic children was significantly higher than that in normal children among both the 120-130 cm (6-10 years) and the 148-158 cm (11-15 years) groups. These results suggest that there may be a strong relationship between airway inflammation and eNO level in children. In elementary school and junior high school, some children diagnosed with asthma experience chronic airway inflammation, even if they participate in everyday school activities without any asthma attacks.

In addition, the eNO level correlated with V25/HT, but not FVC and FEV1 in the younger children and correlated with MMEF and V25/HT in the older children. Among the younger children, the parameter of small respiratory resistance, R5-R20, significantly correlated with the eNO level in the asthmatic group. This tendency was not evident in the non-asthmatic children.

The eNO level in asthmatic children was significantly correlated with small airway parameters. Our data suggest that eNO is mainly produced in small airways in the stable state of childhood asthma, and small airway lung function parameters are good indicators for persistent airway inflammation. Chronic “silent” small airway inflammation, which induces small airway obstruction by smooth muscle constriction and/or mucosal edema, significantly increases the eNO level in asthmatic children, whereas acute constriction of airways may induce a temporal decrease of eNO level as described in previous reports.11-13 The effect of gender on eNO level in children has also been discussed but is still controversial.16,23 In this report, we did not observe any effect of gender on the eNO level in children.

IOS is a relatively new technique for the assess-

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Table 4 Partial Correlation Coefficients: Controlling for Gender

<table>
<thead>
<tr>
<th>Height (cm)</th>
<th>Parameter</th>
<th>FVC</th>
<th>FEV1</th>
<th>MMEF</th>
<th>V50</th>
<th>V25/HT</th>
<th>R5</th>
<th>R5-R20</th>
<th>X5</th>
</tr>
</thead>
<tbody>
<tr>
<td>120-130</td>
<td>Total</td>
<td>CC</td>
<td>-0.017</td>
<td>-0.064</td>
<td>-0.148</td>
<td>-0.107</td>
<td>-0.190</td>
<td>-0.110</td>
<td>-0.126</td>
</tr>
<tr>
<td></td>
<td>(n = 148)</td>
<td>p</td>
<td>0.857</td>
<td>0.488</td>
<td>0.110</td>
<td>0.242</td>
<td>0.037</td>
<td>0.236</td>
<td>0.173</td>
</tr>
<tr>
<td>148-158</td>
<td>Total</td>
<td>CC</td>
<td>0.046</td>
<td>-0.115</td>
<td>-0.183</td>
<td>-0.165</td>
<td>-0.259</td>
<td>0.018</td>
<td>-0.112</td>
</tr>
<tr>
<td></td>
<td>(n = 180)</td>
<td>p</td>
<td>0.568</td>
<td>0.156</td>
<td>0.023</td>
<td>0.041</td>
<td>0.006</td>
<td>0.821</td>
<td>0.880</td>
</tr>
</tbody>
</table>
ment of airway obstruction, and is particularly useful in patients who are not able to perform forced breathing maneuvers.\textsuperscript{24,25} Vink \textit{et al.} demonstrated that R and X values showed significant changes during airflow obstruction by methacholine challenge.\textsuperscript{26} In the present study, the eNO level did not correlate with an increase in large airway resistance (R\textsubscript{20}) or X\textsubscript{s}, but did correlate small airway resistance (R\textsubscript{5-R20}) in younger children. This result supports our above-mentioned conclusion, and indicates a mechanical difference in asthma control between younger and older children.

These data suggest that eNO levels correlate with the degree of small airway bronchoconstriction in asymptomatic asthmatic children. It is possible that the relative reduction of the ventilated airway surface by bronchoconstriction and/or mucosal edema in small airways induces a decrease in eNO, because eNO predominantly originates from the terminal and respiratory mucosa in bronchioles.\textsuperscript{27} Small airway constriction and/or mucosal edema, which cause a reduction of the luminal airway surface,\textsuperscript{28,29} may therefore lead to a decreased eNO level. Moreover, NO trapping in the small airways during bronchoconstriction and/or mucosal edema may contribute to the reductions in the eNO level.\textsuperscript{11} It is possible that eNO production due to airway inflammation in the terminal and respiratory bronchioles was more influential than the effect of bronchoconstriction and/or mucosal edema.

The incidence of asthmatic children in this study was higher than the Japanese standard. Our annual surveys of this area in Gunma Prefecture suggest that the incidence of asthmatic children in this area is higher than elsewhere in Japan. The precise reason for this phenomenon is unknown, but the incidence of familial smoking was about twice as high as elsewhere (about 70% or more), and sometimes photosensory smog is present in the area. In addition, there may have been some bias in the selection of the subjects who knew the reason for our study and, accordingly, participated. However, our study covered a strictly limited area, season and time, and therefore the incidence of asthma does not directly affect our conclusions.

Consequently, chronic “silent” small airway inflammation significantly increases the eNO level in asthmatic children. To our knowledge, this is the first study demonstrating a relationship between small airway narrowing and the level of eNO while controlling for age and height in children, and the relationship between the level of eNO and the small airway parameter, R\textsubscript{5-R20}. Small airway lung function, which is a good indicator of persistent airway inflammation, should be monitored for effective control of childhood asthma. Furthermore, when the eNO level is measured, the effects of both persistent and acute bronchoconstriction and/or mucosal edema in small airways should be considered.

**ACKNOWLEDGEMENTS**

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