SHORT COMMUNICATION

Predicting airway hyperreactivity to mannitol using exhaled nitric oxide in an unselected sample of adolescents and young adults

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Summary
Increased levels of exhaled nitric oxide (FeNO) and airway hyperresponsiveness (AHR) to inhaled mannitol are related to allergic inflammation characterized by eosinophil infiltration and a clinical response to treatment with anti-inflammatory agents in subjects with asthma. This study determines the diagnostic accuracy of FeNO using absolute and normalized values to predict the presence of AHR to inhaled mannitol in an unselected population.

Levels of FeNO and AHR to inhaled, dry-powder mannitol was measured in 180 unselected, steroid-naive, non-smoking adolescents and young adults.

The area under the curve for the receiver operating characteristics curve for FeNO to identify a positive response to mannitol was 91.9% (CI95: 87.7–96.2). The optimal cut-off was 25 ppb (185% predicted) and a sensitivity of 100% (CI95: 83.9–100.0) was achieved below 20 ppb (165% predicted).

FeNO is a sensitive and specific tool for predicting the response to inhaled mannitol in an unselected sample of non-smoking, steroid-naive subjects, and a low FeNO indicates that extra diagnostic work-up using inhaled mannitol will add very little extra information.

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Chronic inflammation of the airways and airway hyperresponsiveness (AHR) are two key pathological features of asthma. The fraction of exhaled nitric oxide (FeNO) is considered an easy and non-invasive marker of airway inflammation. Increased levels of FeNO are closely related to allergic inflammation characterized by eosinophil infiltration and a clinical response to treatment with anti-inflammatory agents such as inhaled corticosteroids (ICS). Inhaled mannitol, a standardized test for AHR with a high diagnostic specificity for asthma, is also suggestive of active inflammation of the airways that will benefit from regular anti-inflammatory treatment. In a selected group of subjects with asthma, raised levels of FeNO correlate with responsiveness to mannitol, and the findings were similar in an unselected group. Both FeNO and mannitol are used regularly as diagnostic tools in the assessment of asthma, and we expect a significant overlap in the properties of the two tests.

The aim of this study was to analyze to what extent FeNO can be used to predict AHR to mannitol, considering that FeNO measurement is easier to perform than mannitol challenge. Furthermore, the diagnostic accuracy of absolute and normalized FeNO in predicting the AHR to inhaled mannitol (% predicted using published reference equations) was compared.

The study is a post hoc analysis on prior published data on subjects randomly drawn from the civil registration list (n = 238) aged 14–24 years (median 19) who had their FeNO (median 15.0 ppb, range 4–263 ppb), lung function and reactivity to mannitol measured. Skin prick tests were performed for the most common aeroallergens. Data on smoking status (51 subjects were current smokers), use of medication (8 were currently on ICS) and respiratory symptoms were obtained through an interview at the time of examination. FeNO levels were measured online (NIOX; Aerocrine AB, Solna, Sweden), according to ATS/ERS recommendations. AHR to mannitol was defined as a decrease in forced expiratory volume in one second (FEV₁) of at least 15% after inhalation of 635 mg of mannitol (% predicted). Skin prick tests were performed for the most common aeroallergens. Data on smoking status (51 subjects were current smokers), use of medication (8 were currently on ICS) and respiratory symptoms were obtained through an interview at the time of examination. FeNO levels were measured online (NIOX; Aerocrine AB, Solna, Sweden), according to ATS/ERS recommendations. AHR to mannitol was defined as a decrease in forced expiratory volume in one second (FEV₁) of at least 15% after inhalation of 635 mg of mannitol or less. Further details about the methods are available in earlier publications.

STATA 12.0 (Stata Corp, TX, USA) was used for the analysis. A receiver operating characteristic (ROC) curve was constructed by plotting levels of FeNO (both absolute and normalized) against a positive mannitol challenge. The area under the curve (AUC) and the optimal FeNO cut-off (defined as the cut-off at which sensitivity and specificity were closest together) were calculated. Normalized levels of FeNO were obtained by using a reference equation based on height from Malmberg et al. and extrapolating to 24 years. The age effect was considered negligible in this sample.

Of 238 subjects included (142 women), 51 were classified as having asthma (based on the assessment of a respiratory specialist blinded to the result of AHR to inhaled mannitol), 76 had allergic rhinitis and 33 had a positive mannitol test (PD₁₅ < 635 mg). Non-atopic, non-smoking, non-asthmatic subjects with no current use of ICS (n = 104) had a mean FeNO% predicted (95%CI) of 101.9 (93.1, 111.6). AUC for absolute values of FeNO was 89.6% (CI95: 84.0–95.2), which means that a subject with a positive mannitol challenge has a 90% probability of having a higher level of FeNO than a subject with a negative mannitol challenge. Using normalized instead of absolute FeNO values increased AUC to 90.6% (CI95: 85.4–95.8), p = 0.046. When current smokers and/or subjects on ICS were excluded, 180 subjects remained and were included in the final analysis. The subgroup of 180 subjects did not differ from the original 238 subjects in terms of sex or atopic status, but the mean age (years) was lower (18.6 vs 18.9, p < 0.01) and fewer had asthma (16.1% vs 21.4, p < 0.01). In the subgroup, there was no significant difference between absolute and normalized FeNO values, AUC being 91.9% (CI95: 87.7–96.2) and 92.4% (CI95: 88.4, 96.4), respectively (Fig. 1). Diagnostic accuracy of FeNO when compared against a positive mannitol challenge test in this subgroup is presented in Table 1.

The results indicate that FeNO is a both sensitive and specific tool for predicting AHR to mannitol in an unselected population of steroid-naïve subjects. The optimal cut-off was 25 ppb (185% predicted). At 20 ppb (165% predicted) the sensitivity was 100% with only a minor concomitant reduction in specificity, implying that at 20 ppb or below AHR to mannitol is very unlikely. The cut-off resembles the recently published American Thoracic Society clinical guidelines on use of exhaled NO suggesting that the presence of eosinophilic airway inflammation is unlikely below 25 ppb. A cut-off of 30 ppb (210% predicted), on the other hand, resulted in a specificity of 90%, which indicates that nine out of ten subjects without AHR to mannitol will present with a FeNO below 30 ppb. At higher values of FeNO, specificity was further increased but with a marked reduction in sensitivity.

Two of the original 238 subjects had a FeNO below 20 ppb and were also responsive to mannitol, but were excluded from analysis due to their status as current smokers, as smoking is associated with reduced FeNO and increased AHR to mannitol. Levels of FeNO and reactivity to mannitol are both decreased during ICS treatment. Excluding subjects that used ICS in our sample did not change the AUC significantly. However, the sample contained few subjects on ICS treatment, and hence we cannot draw conclusions about the usefulness of FeNO for predicting AHR to mannitol in ICS-treated subjects with asthma.

![Figure 1](Image) ROC curves for FeNO absolute values (black circles) and FeNO% predicted values (gray diamonds) to predict AHR to mannitol in non-smoking, steroid-naïve subjects (n = 180).
In our subject sample, highly homogenous e.g. in terms of ethnicity and age, diagnostic accuracy was only minimally improved by using normalized instead of absolute FeNO values. However, cut-offs expressed in % predicted are readily generalized, enabling them to be applied to other groups of patients, for example children.

In conclusion, FeNO is a sensitive and specific tool for predicting the response to inhaled mannitol in an unselected sample of non-smoking, steroid-naive subjects. At a FeNO value below 20 ppb (or approximately 165% predicted) the sensitivity (and negative predictive value) is 100%, which implies that AHR to mannitol can be ruled out with great certainty and that additional diagnostic work-up using inhaled mannitol will add very little extra information. The diagnostic value of FeNO should be further explored in symptomatic subjects with suspected asthma in order to see whether the result of a low FeNO also excludes AHR to mannitol in this group of subjects, and to be able to define clinically relevant predictive values for a diagnosis of asthma.

Conflict of interest statement

Asger Sverrild and Andrei Malinovschi have no financial disclosures. Celeste Porsbjerg has received a fee from Pharmaxis and Nigaard within the past 5 years for speaking. Vibeke Backer has received funds for a staff member from Pharmaxis as well as a fee from Pharmaxis and Nigaard within the past 5 years for speaking. Kjell Alving is an employee and minority shareholder of Aerocrine AB.

References


### Table 1 Predictive values of FeNO (absolute values in Panel A and % predicted values in Panel B) at three cut-offs (100% sensitivity, optimal cut-off, 90% specificity) in non-smoking, steroid-naive subjects 14–24 years old (*n* = 180).

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. FeNO absolute</strong></td>
<td></td>
<td></td>
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<tr>
<td>20 ppb</td>
<td>1.000 (0.839–1.000)</td>
<td>0.748 (0.674–0.814)</td>
</tr>
<tr>
<td>25 ppb*</td>
<td>0.857 (0.637–0.970)</td>
<td>0.836 (0.770–0.890)</td>
</tr>
<tr>
<td>30 ppb</td>
<td>0.714 (0.478–0.887)</td>
<td>0.906 (0.849–0.946)</td>
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<tr>
<td><strong>B. FeNO% predicted</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165%</td>
<td>1.000 (0.839–1.000)</td>
<td>0.818 (0.749–0.874)</td>
</tr>
<tr>
<td>185%*</td>
<td>0.857 (0.637–0.970)</td>
<td>0.836 (0.770–0.890)</td>
</tr>
<tr>
<td>211%</td>
<td>0.714 (0.478–0.887)</td>
<td>0.906 (0.849–0.946)</td>
</tr>
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</table>

*Optimal cut-off corresponding to where sensitivity and specificity are closest together.*