

Combining the Mannitol Test and FeNO in the Assessment of Poorly Controlled Asthma

Celeste Porsbjerg, MD, PhD, Asger Sverrild, MD, PhD, and Vibeke Backer, MD, DMedSci *Copenhagen, Denmark*

What is already known about this topic? The mannitol test and fractional exhaled nitric oxide (FeNO) are increasingly used for diagnosing and phenotyping asthma, but the usefulness of combining the 2 tests has not been evaluated.

What does this article add to our knowledge? In asthmatic patients with insufficient symptom control, the combination of the mannitol test and FeNO enabled the separation into patients with eosinophilic and noneosinophilic asthma.

How does this study impact current management guidelines? In patients with asthma referred for specialist management because of poor symptom control, combining the mannitol test and FeNO may aid in determining the degree and type of underlying airway pathology.

BACKGROUND: International guidelines recommend up-titration of anti-inflammatory treatment in asthmatic patients with poor symptom control, but patients without eosinophilic airway inflammation are less likely to benefit from this. The mannitol bronchoprovocation test and fractional exhaled nitric oxide (FeNO) are increasingly used in the diagnostic assessment of asthma, but the utility of combining these tests has not been evaluated.

AIM: The aim of this study was to determine the value of combining FeNO and the mannitol test to assess patients with asthma referred for specialist assessment because of poor symptom control.

METHODS: All patients referred consecutively over a 12-month period for the assessment of asthma at the Respiratory Outpatient Clinic at Bispebjerg Hospital in Copenhagen were examined with bronchial provocation to mannitol, FeNO, and induced sputum. **RESULTS:** Among asthmatic patients with partly controlled or uncontrolled symptoms according to Global Initiative for Asthma criteria, only 23% had sputum eosinophilia (eosinophils >2.99%). A positive mannitol test did not increase the likelihood of airway eosinophilia significantly (positive test: 32% vs negative test: 18%, $P = .12$). However, a positive mannitol test combined with a FeNO > 25 ppb indicated a high likelihood of airway eosinophilia (73%), compared with FeNO > 25 ppb and

a negative mannitol test (29%) ($P < .05$). In contrast, in patients with FeNO < 25 ppb, a positive mannitol test was not associated with airway eosinophilia (sputum eosinophils > 2.99%: positive mannitol test: 0%, negative test: 11%, ns).

CONCLUSION: Combining the mannitol test and FeNO may aid in the differentiation between eosinophilic and noneosinophilic asthma in patients referred for specialist management because of poorly controlled asthma symptoms. © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;3:553-9)

Key words: Asthma; Phenotype; Eosinophils; Airway inflammation; FeNO; Mannitol

International guidelines on asthma management recommend that treatment be titrated according to the level of symptom control,^{1,2} as asthmatic patients with frequent and severe symptoms are at higher risk of future exacerbations.^{2,3} Accordingly, up-titration of anti-inflammatory treatment is recommended in patients with poor symptom control, whereas treatment may be down-titrated in patients with few symptoms.²

There is however a discrepancy between symptoms and the degree of airway pathology, as patients with well-controlled asthma symptoms may have significant airway hyper-responsiveness (AHR) and significant airway inflammation.^{4,5} Conversely, some patients are highly symptomatic in the absence of objective evidence of airway pathology,⁴ and may be at risk of up-titration of treatment and ultimately of being classified as severe, treatment refractory asthma, based on their lack of response to high dose anti-inflammatory treatment.⁶⁻⁸

Hence, it may be advocated to combine the assessment of asthma symptom control with objective markers of airway inflammation and AHR, particularly in highly symptomatic patients not responding to an initial treatment. This is especially relevant in a specialist setting, where patients are typically referred because of a nonsatisfactory response to treatment by the general practitioner.

In patients with asthma in whom symptoms reflect underlying airway eosinophilia, up-titration of anti-inflammatory treatment

Respiratory Research Unit, Department of Respiratory Medicine, Bispebjerg University Hospital, Copenhagen, Denmark

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Corresponding author: Celeste Porsbjerg, MD, PhD, Respiratory Research Unit, Department of Respiratory Medicine, Bispebjerg University Hospital, Bispebjerg Bakke 23, 2400 Copenhagen NV, Denmark. E-mail: celeste.porsbjerg@regionh.dk. 2213-2198

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Abbreviations used

AHR- Airway hyperresponsiveness
 RDR- Response dose ratio
 FeNO- Fractional exhaled nitric oxide
 ICS- Inhaled corticosteroid
 LABA- Long-acting beta 2-agonist
 PEF- Peak-flow variability
 SABA- Short-acting beta 2-agonist

is likely to result in improvement of symptoms as well as a reduced risk of exacerbations,^{9,10} whereas patients with non-eosinophilic asthma are less likely to respond to anti-inflammatory treatment.¹¹⁻¹⁴ Determining the inflammatory phenotype of asthma may therefore be clinically relevant, to predict a response to treatment.

Measurement of sputum eosinophilia is difficult and time-consuming, which has limited its implementation in the daily clinical setting outside specialist clinics with specific interest, where diagnostic tests need to be practically feasible, easily performed, and provide instantly readable results.

Fractional exhaled nitric oxide (FeNO) is a more feasible method for assessing airway eosinophilic inflammation.^{15,16} It has practical advantages such as being fast and easy to perform in most patients, but may be limited by a lack of specificity as it may be elevated in patients with allergic rhinitis or respiratory infection, and is suppressed in smokers.¹⁶⁻¹⁸

The mannitol bronchial provocation test is an indirect challenge test, which is increasingly used in the diagnostic assessment of asthma.^{19,20} The response to mannitol is related to the degree of airway eosinophilia.²¹ Hence, patients with a positive test are more likely to have airway eosinophilia, and conversely, patients not responding to mannitol are unlikely to have significant eosinophilia.^{21,22} However, patients with noneosinophilic asthma may also respond to the mannitol test,^{21,23} and hence the test alone will not differentiate eosinophilic asthma from noneosinophilic asthma. Furthermore, the association between the response to mannitol and the level of eosinophilic airway inflammation has so far only been studied in selected patient groups, and may be weaker in more general, nonselected clinical populations.

An outstanding question is whether FeNO and the mannitol test may be combined to supplement the symptom-based assessment of the degree of asthma control, to inform a treatment decision on up- or down-titration of treatment. Furthermore, as new treatments targeting specific pathways in asthma emerge, tools that may aid in phenotyping asthma in the daily clinical setting become increasingly important.

We hypothesized that the combined use of AHR to mannitol and FeNO may enable differentiation between patients with and without significant airway eosinophilia, among patients with poor control. Hence, the present study analyzes data from the MAPOut II study, a real life study of a population of patients referred for specialist management of asthma, over a 12-month period, and evaluates the ability of FeNO and AHR mannitol to assess poor symptom control relating to eosinophilic airway inflammation.

METHODS AND MATERIAL

The MAPOut II study is a cross-sectional study of patients referred for specialist management of asthma. With the aim of describing a clinically representative population of patients assessed for asthma in a

specialist setting, we recruited among all patients referred for specialist assessment over a 12-month period for either suspected asthma or known asthma, with few exclusion criteria: respiratory diseases other than asthma (eg, sarcoidosis, chronic obstructive pulmonary disease), age < 15 years, individuals older than 40 years with a smoking history of more than 10 pack-years, pregnancy, and recent respiratory infection (<6 weeks). Patients younger than 40 years with asthma symptoms and tobacco consumption more than 10 pack-years were eligible for the study (Figure 1, consort statement). Patients were enrolled between March 2011 and April 2012.

A total of 246 subjects were referred for the assessment of known or suspected asthma, over the 12-month study period. As described in the consort statement, a total of 190 patients participated in the study, equivalent to 80% of the population eligible for inclusion in the study (Figure 1). Of these 190 subjects, 151 were concluded to have asthma, in accordance with the predefined criteria.

In the present analysis of FeNO and mannitol in the assessment of poorly controlled asthma, only asthmatic patients with either partly controlled or uncontrolled asthma according to Global Initiative for Asthma (GINA) criteria were included (n = 133, Figure 1). A representative sputum sample was obtained in 105 patients (105/133: 79%), FeNO measures were obtained in 99 patients, and a mannitol provocation was performed in 97 patients; 91 patients had all 3 successfully performed.

Definition of asthma. Asthma was defined as symptoms suggestive of asthma, *as well as either* at least one objective marker of variable airflow obstruction (reversibility to beta-2-agonist [$\geq 12\%$ and at least 200 mL] or peak-flow variability [PEF (max-min/max) $\times 100 \geq 20\%$]), AHR to either mannitol (PD15 ≥ 635 mg) or methacholine (PD20 ≥ 8 μ mol), *or* a doctor's diagnosis of asthma and current use of inhaled corticosteroids, as these patients could have a negative test panel, in spite of having asthma.

The study was approved by the local ethical committee (H-3-2011-121), and all subjects signed an informed consent.

Participants were assessed with a 3-day asthma evaluation program, over 2 weeks, of which the first visit included spirometry with a reversibility test after 4 puffs of terbutaline (2 mg). On the second visit, the participants had FeNO measured, a skin prick test with 10 standard allergens, and a methacholine challenge test. At the last visit, responsiveness to inhaled mannitol was measured, and sputum induction was performed. All participants were interviewed using a questionnaire based on the GINA guidelines, and answered a number of questionnaires including Asthma Control Questionnaire (ACQ) and mini Asthma Quality of Life Questionnaire (mini-AQLQ).²⁴ In the 2-week period between the first and the last visit, PEF variability was monitored (morning and evening).

Definition of asthma control. Asthma symptom control was assessed according to the GINA 2010 guideline recommendations²: daytime symptoms (>twice weekly?), nighttime symptoms (any?), need for reliever medications (>twice weekly?), limitations due to asthma (any?), exacerbation(s) during the past 12 months, and a forced expiratory volume in 1 second (FEV1) < 80% of predicted. Partly controlled asthma was defined as having 1-2 of these components, and uncontrolled asthma as having 3-4.

Skin prick test. A skin prick test was performed with a panel of allergen extracts, including birch, grass, mugwort, horse, cat, dog, *Dermatophagoides pteronyssinus* and *D. farinae*, Alternaria, and

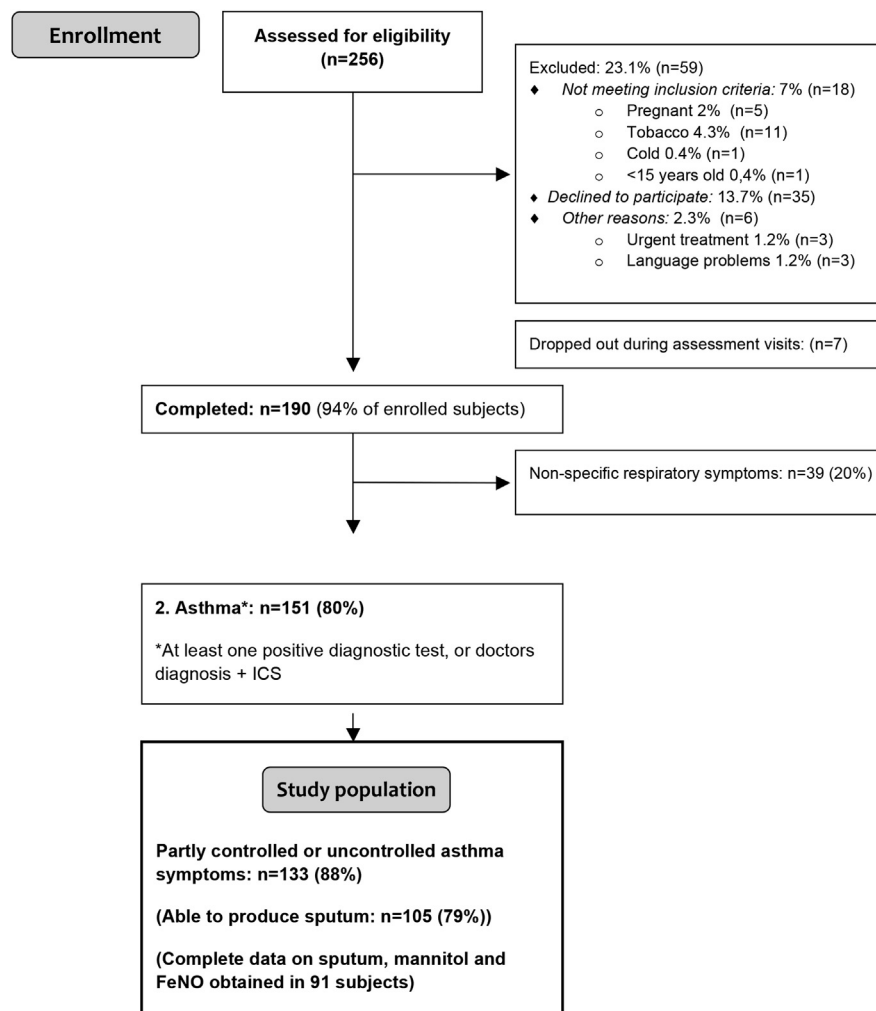


FIGURE 1. Strobe flow diagram describing the study population: all patients referred for specialist management of suspected or known asthma over a 12-month period were screened, and eligible subjects were offered participation. The figure describes the proportion of eligible patients who were enrolled, as well as the proportion completing the study. In the present analysis, only those patients who were concluded to have asthma, and who had incomplete asthma control, were included.

Cladosporium herbarum.²⁵ A positive response (atopy) was calculated with a cutoff value of 3 mm.

Fractional exhaled nitric oxide. Fractional exhaled nitric oxide (FeNO) was measured with the Niox Minor (Aerocrine AB, Solna, Sweden), according to the American Thoracic Society (ATS) practice guidelines.¹⁶ In accordance with the European Respiratory Society (ERS) ERS/ATS recommendations, a low FeNO level was defined as <25 ppb, an intermediate level as 25–50 ppb, and a high level as >50 ppb.¹⁶

Spirometry. Lung function was done using maximum expiratory flow volume according to the standards specified by the ERS and ATS (Miller), using a MasterScreenPneumo spirometer (Jaeger). A reversibility test was performed using 4 puffs of 0.5 mg terbutaline, followed by lung function measurement after 15 minutes.²⁶ A significant reversibility was defined as a 12% increase in FEV₁ (and minimum 200 mL). Predicted normal values based on sex, height, weight, and age for FEV₁, forced vital capacity (FVC), FEV₁/FVC ratio were calculated using NHANES reference values.²⁷

Peak-flow variability. All patients were asked to measure PEF for 2 weeks, in the morning and in the evening. Variation was measured as the highest PEF minus the lowest PEF divided by the highest. A significant variability was defined as a day-to-day variation of at least 20% ((maximum–minimum)/maximum).

Mannitol bronchial provocation. The participants inhaled an empty capsule followed by capsules with increasing doses of mannitol (from 5 to 635 mg) until maximum doses have been reached or achieving a 15% reduction in FEV₁ (PD15).¹⁹ Patients with a cumulative dose of mannitol of ≤635 mg and a decrease in FEV₁ ≥ 15% were defined as having a positive test (AHR). For subjects with a positive test, the sensitivity to mannitol was calculated as the dose resulting in a 15% decrease in FEV₁ (PD15). For all subjects, the degree of responsiveness was calculated at the response dose ratio (RDR): percent fall in FEV₁/mg mannitol administered.

Methacholine bronchial provocation. A dosimetric method was used to measure airway responsiveness to inhaled

TABLE I. Patient characteristics

	n = 133
Gender (% female)	59%
Age (median, min–max)	26 (15–59)
BMI	22.7 (±1.1)
Atopy*	58%
Allergic rhinoconjunctivitis	79%
Smoker (current/ex)	8% (34%)
Pack-years	3.1 (1.2)
ICS use (ICS/LABA use)	52% (23%)
ICS dose (budesonide equivalent, µg/day)	350 (±450)
Asthma control (partly controlled/uncontrolled)	41%/59%
ACQ-5 score	1.5 (±1.0)
AQLQ score	5.5 (±1.0)
Exacerbation (last 12 mo)	42%
Exacerbation treated with prednisone (last 12 mo)	11%
Emergency visit due to exacerbation (last 12 mo)	15%
FEV1% (pre-SABA)	92.0 (±3.0)
FEV1% (post-SABA)	97.2 (±3.4)
FEV1/FVC (pre-SABA)	0.81 (±0.02)
FEV1 < 80%	21%
Reversibility (%)	5.6% (±0.9)
FeNO (ppb) (median, min-max)	18.5 (3–184)
FeNO <25 ppb/25–50 ppb/>50 ppb	64%/25%/11%
Sputum eosinophils > 2.99%	24%
Sputum eosinophils (%), geometric mean (CI)	1.4 (1.1–1.8)
AHR to mannitol	36%
PD15 (mg), geometric mean (CI)	194 (125–316)
RDR to mannitol (%fall in FEV1/mg), geometric mean (CI)	0.05 (0.01–0.34)
AHR to methacholine	64%
PD20 (µmol), geometric mean (CI)	1.1 (0.6–1.5)
RDR to methacholine (%fall in FEV1/mg), geometric mean (CI)	15.8 (4.3–58.8)

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FeNO, fractional exhaled nitric oxide; AHR, airway hyperresponsiveness; RDR, response dose ratio; FVC, forced vital capacity; LABA, long-acting beta-2 agonist; RDR, response dose ratio; SABA, short-acting beta 2-agonist.

Continuous variables are given as mean (SD) unless otherwise stated.

*Positive skin prick test to the standard inhalation panel.

methacholine. A Jaeger dosimeter generated the aerosols in the test solution (Jaeger GmbH, Germany). The patient inhaled a dose of isotonic saline, followed by 5 successively increasing doses of methacholine ranging from 0.06 to 7.8 µmol, until a cumulated dose of 7.8 µmol or a 20% decrease in FEV₁ was reached. Patients with a cumulative dose of methacholine of ≤7.8 µmol and a decrease in FEV₁ ≥ 20% were defined as having a positive test (AHR). For patients with a positive test, the sensitivity to methacholine was calculated as the dose resulting in a 20% decrease in FEV₁ (PD₂₀). For all patients, the degree of responsiveness was calculated at the RDR: percent fall in FEV₁/µmol methacholine.

Sputum induction. Sputum induction was conducted according to ERS recommendations [18]. After inhalation of 1 mg terbutaline, sputum was induced by inhalation of hypertonic saline in increasing concentrations (3%, 4%, and 5%) for 3 time periods each of 6 min (total duration 18 min). Sputum plugs were selected and

processed, cytopins were prepared using standard methods, and a differential cell count was performed.²⁸

Statistical analysis

Statistical analysis was performed with SPSS, version 20.0. Continuous variables were reported as mean (SD) for normally distributed data. RDRs to mannitol and methacholine, FeNO, and % sputum eosinophils were log-normally distributed, and therefore log-transformed to enable use of parametric statistical methods, and described with the geometric mean (CI). ANOVA tests and unpaired t-tests were used for comparison of continuous variables, and Cochran-Mantel-Haenszel and Fisher's exact test for categorical variables. The correlation between Log% sputum eosinophils and LogRDR to mannitol was analyzed with Pearson's correlation analysis.

RESULTS

The median patient age was 26 years (15–59 years), with the majority of patients being nonsmokers (92%) and normal weight (mean BMI 22.7). Most patients had a well-preserved lung function; only 21% had an FEV₁ < 80% of predicted, and the mean reversibility was 5.6% (Table I). The mean ACQ score was 1.5 (±1.0), and the AQLQ score was 5.5 (±1.0), which showed that these patients, in general, were relatively symptomatic.

A successful sputum sample was induced in 105 subjects (79%), of whom, 24% had eosinophilic airway inflammation (sputum eosinophils > 2.99%). Patients with uncontrolled asthma symptoms tended to be slightly more likely to have airway eosinophilia than partly controlled patients, although the difference was not statistically significant (32% and 19%, respectively, $P = .16$).

Overall, AHR to mannitol was not associated with an increased likelihood of airway eosinophilia; 32% of those with a positive mannitol test had airway eosinophilia, compared with 18% of those not responding to the test ($P = .12$) (Table II). However, patients with moderate to severe AHR to mannitol (ie, a PD₁₅ < 155 mg) were more likely to have airway eosinophilia compared with subjects not responding to mannitol (44% vs 18%, $P = .03$). Patients with a mild degree of AHR to mannitol did not differ from patients not responding to mannitol (22% vs 18%, $P = .8$).

The level of FeNO predicted the likelihood of airway eosinophilia: among patients with a low FeNO (<25 ppb), only 10% had airway eosinophilia, whereas the proportion increased to 30% in patients with an intermediate FeNO (25–50 ppb) and 83% in those with a FeNO > 50 ppb ($P < .05$) (Table II).

The main aim of this analysis was to evaluate the combined use of FeNO and the mannitol test. When combining the test results, patients with low FeNO were found to be unlikely to have airway eosinophilia, regardless of the result of the mannitol test (11% vs 0%, ns) (Figure 2). In contrast, in patients with a high FeNO (>25 ppb), AHR to mannitol was associated with a significantly higher likelihood of airway eosinophilia than a negative test (73% vs 29%, $P < .05$) (Figure 2).

Accordingly, the highest level of sputum eosinophils was observed in patients with a high FeNO in combination with a positive mannitol test (sputum % eosinophils (geometric mean (CI): positive test: 5.6% (2.2–13.9), negative test: 2.0% (1.3–3.2); $P = .02$), whereas among patients with a low FeNO, the percentage of sputum eosinophils was low in both mannitol positive and mannitol negative subjects (positive test: 0.6% (0.5–0.9), negative test: 1.0% (0.7–1.2); $P = .07$). This indicates that in the absence of an elevated FeNO, a positive mannitol test does not indicate eosinophilic airway inflammation.

TABLE II. Airway eosinophils according to the level of FeNO and AHR to mannitol among patients with partly controlled or uncontrolled asthma*

	Mannitol test		FeNO		
	Negative (n = 61)	Positive (n = 36)	<25 ppb (n = 61)	25-50 ppb (n = 26)	>50 ppb (n = 12)
Sputum eosinophils, Geometric mean (CI)	1.3 (1.0-1.6)	1.68 (0.9-3.0) ^{ns}	0.9 (0.8-1.1)	1.8 (1.1-2.8)	9.3 (4.2-20.1) [†]
Sputum eosinophils > 2.99%	18%	32% ^{ns}	10%	30% [†]	83% [†]

FeNO, fractional exhaled nitric oxide; AHR, airway hyperresponsiveness.

% sputum eosinophils: unpaired t-test (mannitol), ANOVA (FeNO).

Sputum eosinophils < 2.99%: Fisher's exact test.

*According to Global Initiative for Asthma guidelines.

[†]P < .001.

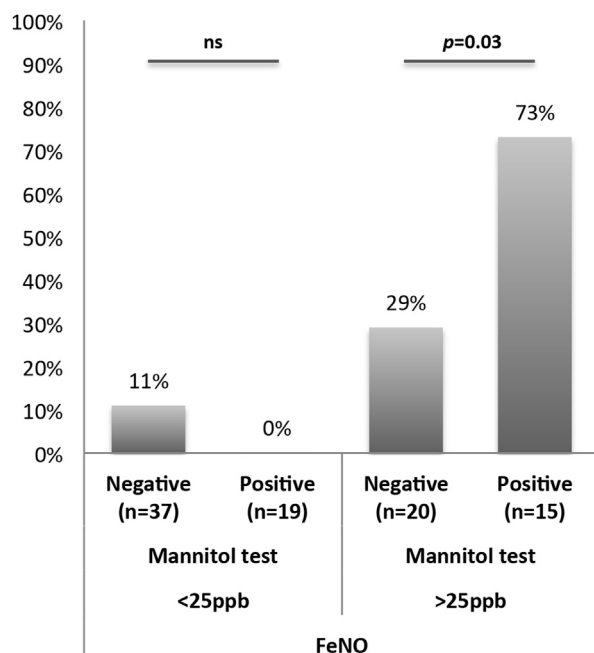


FIGURE 2. Proportion of asthmatic patients with airway eosinophilia (>2.99%), according to the level of fractional exhaled nitric oxide (FeNO) and response to the mannitol test: in asthmatic patients with a low FeNO (<25 ppb), a positive mannitol is not indicative of eosinophilic airway inflammation, whereas in patients with elevated FeNO (>25 ppb), a positive mannitol test indicates a significantly higher likelihood of airway eosinophilia, compared with mannitol negative patients.

In keeping with these observations, the response to mannitol correlated with the degree of eosinophilic airway inflammation in subjects with FeNO > 25 ppb (Pearson correlation coefficient: 0.38, $P = .02$) but not in those with FeNO < 25 ppb (-0.13 , $P = .35$) (Figure 3).

An analysis of the 3 FeNO strata (<25 ppb, 25-50 ppb, and >50 ppb) showed that among patients with a high FeNO (>50 ppb), all patients with a positive mannitol test had sputum eosinophilia, compared with half of those with a negative test (100% vs 50%, $P = .04$), whereas in the intermediate FeNO group (25-50 ppb), the difference was not statistically significant (50% vs 26%, $P = .06$). However, this analysis was limited by the very few patients in each subgroup (eg, in the >50 ppb group [$n = 11$], 7 of whom had a negative mannitol test), which

prohibits any firm conclusions on the significance of a positive mannitol test in the intermediate group.

DISCUSSION

The present study examined the value of adding objective markers to the assessment of asthma symptom control, in patients with asthma referred for a specialist assessment. We found that the majority of patients with poorly controlled symptoms did not have underlying eosinophilic airway inflammation. The use of a combination of FeNO and mannitol enabled the differentiation between patients with and without significant eosinophilic airway inflammation, and may be used to guide asthma management decisions. When combining FeNO and the response to mannitol, we saw that in patients with a low FeNO, eosinophilic inflammation was very unlikely, regardless of whether the patient responded to mannitol or not, whereas in patients with an elevated FeNO, AHR to mannitol was a strong indicator of eosinophilic airway inflammation. We also applied the cutoff values suggested by the ATS practice guidelines on FeNO, and found that in this real life patient population, the predictive values of a low, intermediate, and high FeNO for airway eosinophilia were in keeping with these recommendations.¹⁶ All patients with a high FeNO and positive mannitol test had airway eosinophilia, whereas the predictive value of a positive mannitol test in the intermediate FeNO group did not quite reach statistical significance. This most likely reflects that when combining the 3 FeNO strata and mannitol test results, the sample size is too small in this study.

Notably, almost half of patients with asthma who reported poorly controlled symptoms did not have an elevated FeNO or AHR to mannitol, which indicated that a substantial number of patients would have been over treated if a treatment decision was based on symptoms alone.²

Accordingly, combining the results of FeNO and the mannitol test appears to be useful in certain patients; patients with a positive mannitol test and an elevated FeNO are likely to have airway eosinophilia, which suggests that these patients should always be up-titrated in anti-inflammatory treatment. In contrast, patients with an elevated FeNO but negative mannitol test are less likely to have underlying eosinophilia, and management decisions should depend on the overall clinical picture.

The mannitol test is an indirect test that acts via the release of bronchoconstricting mediators from inflammatory cells in the airways.^{19,29,30} Accordingly, the mannitol test has been shown to have a closer relationship to the level of eosinophilic airway inflammation than direct tests such as methacholine.^{21,22} We have previously reported that in a population study setting, a low

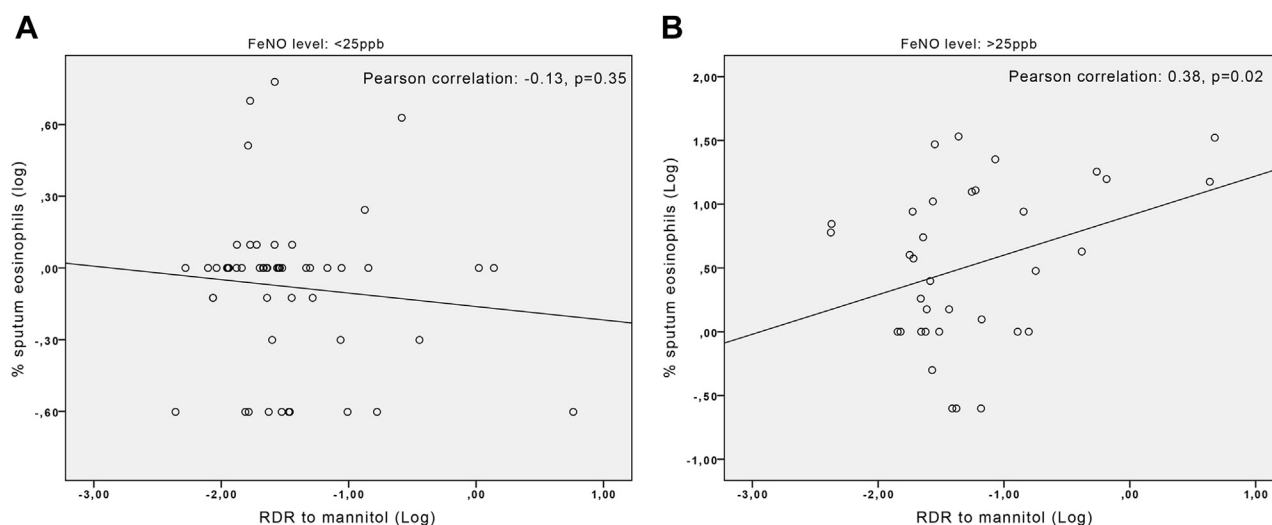


FIGURE 3. Scatterplot of responsiveness to mannitol vs % sputum eosinophils in patients with (A) fractional exhaled nitric oxide (FeNO) < 25 ppb and (B) >25 ppb, illustrating that in asthmatic patients with low FeNO, there is no association between the level of airway eosinophilia and the degree of airway hyperresponsiveness (AHR), whereas in patients with elevated FeNO, the degree of AHR is correlated to the degree of eosinophilic airway inflammation.

FeNO may preclude the need for a mannitol test, as few will respond to mannitol.³¹

In contrast, in the present clinical asthmatic population, we found that half of asthmatic patients with AHR to mannitol did not have eosinophilic inflammation. This is a higher proportion than previously reported in selected, untreated asthmatic patients,²¹ but similar to inhaled corticosteroid (ICS)-treated patients.³² However, we did not find that AHR to mannitol in patients with noneosinophilic asthma was explained by ICS use, nor by smoking (data not shown). In keeping with the present findings, we have recently reported that in a private specialist setting, 26% of asthmatic patients with AHR to mannitol had a low FeNO,³³ which suggests that patients referred to a specialist are more likely to have atypical or noneosinophilic asthma.

At present, the best management strategy in noneosinophilic asthma has not been clarified. AHR to mannitol in noneosinophilic asthma may relate to mast cell myositis. The response to mannitol has been shown to relate to the release of bronchoconstricting mediators such as leukotriens and prostaglandins primarily released from mast cells.^{29,34} Mast cell myositis has been demonstrated to be equally common in non-eosinophilic and eosinophilic asthma,¹¹ and may accordingly also drive AHR to mannitol in noneosinophilic asthma. ICS treatment may reduce mast cell infiltration, but requires higher doses, as demonstrated in the EOS bronchoscopy study, where a fixed dose ICS/long acting beta-2 agonist (LABA) combination that contained a high dose of budesonide (1600 µg/day) resulted in a significant reduction of mucosal mast cells, as opposed to a variable dose of budesonide (mean dose: 640 µg/day).³⁵ This may explain why randomized studies of the effect of ICS in noneosinophilic asthma have shown varying results: whereas mometasone dosed at 400 µg/day did not improve symptoms or AHR to methacholine in a bronchoscopy study by Berry et al,¹¹ high dose ICS treatment with fluticasone (1000 µg/day) improved asthma control as well as indirect AHR to AMP.¹³

More than half of patients with poorly controlled symptoms had a negative mannitol test and low FeNO. These are unlikely

to have airway eosinophilia, which suggests that these patients should not be up-titrated in anti-inflammatory treatment, in spite of poorly controlled symptoms. No randomized studies have so far evaluated this patient group, but studies of patients with good symptom control have shown that ICS may be successfully down-titrated in patients with a negative mannitol test.^{36,37}

The main limitation of this study is that as a real life study, broad inclusion criteria were applied. Hence, the patients included do not reflect those in a typical randomized clinical trial (RCT studies), but may have milder or more atypical disease, as well as more exposure to factors such as smoking or recurrent infections. Conversely, the strength of this study is that it demonstrates the advantages as well as the limitations of FeNO and the mannitol test in an unselected clinical cohort of patients with asthma, which makes the results applicable in a specialist clinical setting.

In spite of having poorly controlled symptoms, most patients in this study had relatively well-preserved lung function and a low level of AHR, and it could be questioned whether this reflects a typical specialist population. On the other hand, patients referred to a specialist setting are recognized to have more discordance between symptoms reported and underlying airway pathology, compared with patients with asthma in primary care.⁴ This underlines the need for objective assessment of asthma severity in this setting. Concordantly, the recent ERS/ATS guidelines on severe asthma recommend a systematic evaluation of patients with uncontrolled symptoms despite high intensity treatment, to differentiate between patients with true refractory asthma and other causes of poor symptom control.^{6,7} These findings support that not only do patients with severe asthma need systematic evaluation, but so do asthmatic patients with moderate disease.

We used absolute, unadjusted FeNO values for defining high versus low FeNO levels, as these are directly applicable in the clinical setting. Although a number of factors can affect FeNO, for example, age, male sex, height, and atopy increase FeNO, and

smoking decreases FeNO,¹⁶⁻¹⁸ there is at present no consensus on how to adjust for these factors,¹⁶ and predicted values based on these factors have not been found to be superior for detecting steroid responsive disease.³⁸

In conclusion, we found that in patients with asthma referred for specialist assessment, only a minority of patients with poor controlled symptoms had underlying airway eosinophilia. By combining FeNO and the mannitol test, patients with eosinophilic airway inflammation could be differentiated from patients without eosinophilia. A significant proportion of patients had AHR to mannitol in the absence of airway eosinophilia, and further studies are warranted to identify the best treatment strategy in these patients with noneosinophilic asthma.

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