

DIAGNOSIS

FENO and AHR mannitol in patients referred to an out-of-hospital asthma clinic: a real-life study

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Abstract

Objective: Ongoing airway inflammation measured by fractional exhaled nitric oxide (FENO) and airway hyperresponsiveness (AHR) to mannitol are associated in selected asthma patients, but no evidence exists of this association in unselected asthma patients. The aim was to investigate the association between FENO and AHR to mannitol in unselected individuals with possible asthma. **Methods:** A real-life study on patients with possible asthma referred to a specialized asthma clinic. Data on asthma history, FEV₁, FENO, atopy, smoking, treatment and AHR to mannitol were collected. **Results:** In 217 unselected patients with symptoms suggestive of asthma, FENO and response to mannitol were tested. Of the 141 who underwent both tests, 32 (23%) had FENO >25 ppb, and 58 (41%) had AHR to mannitol. A significant association between high FENO and AHR was found ($p < 0.001$); 26% responded to mannitol despite a normal NO, and 8% had a high FENO but no AHR. Additionally, a weak association was found between log FENO and log response to mannitol ($r = 0.32$, $p < 0.01$). The area under the ROC curve for FENO as a predictor of AHR was 0.66 (95% CI 0.6–0.8) and for mannitol for having high FENO was 0.73 (95%CI 0.6–0.9). **Conclusion:** In a large sample of patients referred to an asthma clinic, an association was found between FENO and AHR to mannitol. However, a significant proportion of asthma patients had a normal FENO despite having AHR, suggesting that in some patients, AHR to mannitol is not driven by eosinophilic airway inflammation.

Keywords

Diagnostics, mechanisms, phenotypes

History

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Introduction

Asthma is the most frequent chronic illness in adolescents and young adults in western societies [1]. However, despite good access to specialist care, asthma is often under-diagnosed and undertreated, possibly relating to difficulties in diagnosing the disease [2].

Respiratory symptoms and signs of reversible obstructive airways characterize asthma, but the clinical diagnosis is a puzzle, both in primary and secondary care, thus there is a need for diagnostic tools that are straightforward, standardized and simple to use. The current diagnostic tools include various questionnaires, physiological measurements and biomarkers [3]. Although a diagnosis of asthma can be symptom-based only according to Global Initiative of Asthma (GINA), the additional use of objective tests is recommended [4].

Fractional exhaled nitric oxide (FENO) has been shown to be a sensitive diagnostic tool for asthma in selected patients in tertiary care [5]. FENO correlates to sputum inflammatory phenotype [6] and is regarded as a biomarker for eosinophilic bronchial inflammation in asthma; however, less evidence is available on unselected cohorts of asthma patients. Other frequently used diagnostic tools in asthma are bronchial

challenge tests to assess airway hyperresponsiveness (AHR). Bronchial challenge testing can be performed with a range of stimuli, including exercise, hyperventilation, hypertonic saline, methacholine or histamine. Nevertheless, many of these tests require elaborate equipment and calibration, making them less feasible to use outside the hospital setting. The mannitol challenge test has the advantage of being a simple, single-use test kit, which has made bronchial challenge testing available to a broader range of clinical settings. The test has a high specificity for asthma [7] and responsiveness to mannitol is suggestive of active inflammation of the airways that will benefit from regular anti-inflammatory treatment [8]. The high specificity of the mannitol test is thought to relate to its mechanism of action via the release of bronchoconstrictive mediators from inflammatory cells in the airways and there is a close relationship between AHR to mannitol and ongoing eosinophilic airway inflammation in sputum as well as FENO [6].

A close association between AHR to mannitol and FENO has also been found in an unselected sample of asthmatic and non-asthmatic subjects [9], indicating that FENO may predict responsiveness to inhaled mannitol [10]. However, in non-selected subjects with asthma, there is currently little evidence of the diagnostic validity of mannitol and its relation with FENO as a marker of eosinophilic airway inflammation [11]. International guidelines recommend both FENO and bronchial challenge tests as diagnostic tools for asthma [3],

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but there are no clear guidelines on when to use these tests [12]. With the implementation of FENO and the mannitol test in out-of-hospital settings, there is a need to examine their clinical validity as well as the association between them and whether both are needed in a clinical setting. The purpose of the study was to evaluate the association between FENO and AHR to mannitol in unselected subjects with possible asthma referred for assessment in an out-of-hospital specialized asthma clinic.

Materials and Methods

Design

A retrospective real-life study of all patients presenting with respiratory symptoms suggestive of asthma ($n = 348$) referred to an out-of-hospital specialized asthma clinic over 36 months. To minimize the number of patients with chronic obstructive pulmonary disease (COPD) in the final analysis, only subjects aged 45 years or younger ($n = 217$, 63%) were included. Complete data on FENO ($n = 199$, 91%), mannitol challenge ($n = 148$, 68%) and atopy ($n = 189$, 87%) were available in 131 patients (60%). The study was approved by the local scientific ethics committee (J.no H2-2012-FSP51).

Materials

Patients: All patients aged between 10 and 45 years referred to a specialized asthma clinic because of persistent respiratory symptoms suggestive of asthma were included over 36 months.

Methods

An asthma specialist conducted all interviews and an experienced respiratory nurse conducted all tests. The interview included information on respiratory disease, allergy, family history of atopic diseases and seasonal variation in lung and nasal symptoms. Lastly, information on all use of medication to treat asthma, rhinoconjunctivitis and/or allergy was obtained. Severity of asthma was classified according to the GINA guidelines as well controlled, partly controlled or uncontrolled [13]; severity of rhinitis was classified according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines [14]. Tobacco consumption was recorded, and patients were classified as never smokers, ex-smokers or smokers; the average number of pack-years was calculated for both smokers and ex-smokers [(average number of cigarettes*years)/20].

Lung function measurements. Lung function was measured using maximum expiratory flow volume according to the standards specified by the ERS and ATS [15], using EasyOne (Spiropharma®, Copenhagen, Denmark).

Mannitol bronchial provocation. The participants inhaled an empty capsule followed by increasing doses of mannitol (from 5 to 635 mg) until the maximum dose had been administered or a 15% reduction in FEV1 was achieved. AHR is reported as either PD15 or as the response-dose rate (RDR) defined as the percentage fall in FEV1 divided by the cumulative dose of mannitol [16].

Skin prick test. The skin prick test was performed according to European standards with a panel of allergen extracts including: birch, grass, mugwort, horse, cat, dog, *Dermatophagoides pteronyssinus* and *D. farinae*, *Alternaria* and *Cladosporium herbarum* [15]. A positive response (atopy) was calculated with a cut-off value of 3 mm.

FENO. Forced expiratory nitric oxide (FENO) was analyzed using equipment from Aerocrine (NioxMinor®, Solna, Sweden) following the recommendations of the ERS and ATS [17]. An increased level of FENO, indicating airway inflammation, was defined as a value higher than 25 ppb [17].

Statistics

The data were analyzed with the statistical software SPSS version 19.0 (SPSS Inc., Chicago, IL). Mean and standard deviations ($\pm$ SD) were calculated for the normally distributed data and median and interquartile ranges were used to describe the skewed distributed data. Geometric mean (GM) was used for log transformation and McNemare paired tests were used to analyze the dichotome response to both FENO and mannitol in the same subjects. To determine how FENO predicts AHR to mannitol, the area under the receiver operating characteristic (ROC) curve was calculated. For the continuous variables, data were analyzed by the analysis of variance (ANOVA) followed by the two-sample *t*-test to compare the groups or paired *t*-test to compare changes within the group. The chi-squared test for unpaired data was used.

Based on the results of AHR to mannitol and FENO, patients were divided into four groups before analysis: (1) low FENO (<25 ppb) [9] and no AHR to mannitol, (2) low FENO and AHR to mannitol, (3) high FENO and no AHR to mannitol and (4) high FENO and AHR to mannitol. Values of $p < 0.05$ were considered significant.

Results

The basic characteristics of the 217 patients aged between 10 and 45 years referred because of persistent respiratory symptoms suggestive of asthma and tested with either/both FENO and/or mannitol are presented in Table 1. Of the 217 patients, 52 had high FENO >25 ppb and 60 had AHR to mannitol with a PD15 <635 mg. Those who underwent only one test and those who underwent both tests differed only in

Table 1. Basic variables (mean (SD)) in the group of unselected patients with respiratory symptoms suggestive of asthma.

	Total	Tested with both mannitol and FENO
Number of patients	217	141
Sex (females)	123 (57%)	83 (59%)
Age (years)	29.1 (9.7)	28.3 (9.8)
Smokers		
Current	16 (8%)	6 (4%)
Ex-smokers	33 (17%)	27 (19%)
Atopy	185 (85%)	127 (90%)
FEV1 %pred	95% (16%)	97% (16%) ^a
FVC % pred	102% (15%)	102% (16%)
FEV1/FVC%	80% (9%)	81% (9%)

^a $p = 0.05$.

respect to a slightly higher FEV1 predicted in those undergoing both tests (Table 1). Of the 141 subjects with data from both tests, 32 (23%) had a FENO >25 ppb, and 58 (41%) had a positive mannitol test. Although there was a significant association between the responses to the two tests ($X^2=10.3$, $p<0.001$), there was a considerable dissociation between responses (Table 2). Only 21 (15%) had positive responses to both tests and 72 (51%) had negative responses to both tests ($p<0.001$, McNemar paired test, Table 1), whereas 26% responded to mannitol despite a normal NO, and 8% had a high FENO but no AHR to mannitol.

A weak but significant association was found between log FENO and log RDR mannitol ($r=0.32$, $p<0.01$, data shown as absolute values in Figure 1). Subjects with a high FENO also had a significantly higher log RDR compared with the group with low FENO [1.89 (0.8) versus 1.47 (0.7), $p<0.01$]. The level of FENO among those with AHR to mannitol was also significantly higher compared with those without AHR to mannitol [27 (28) versus 20 (18) ppb, $p=0.01$]. Lastly, the area under the ROC curve for FENO as a predictor of having AHR to mannitol was 0.66 (95%CI 0.6–0.8) and for log RDR for having high FENO (>25 ppb) was 0.73 (95%CI 0.6–0.9).

Univariate analyses of the four groups (low FENO/–AHR, low FENO/+AHR, high FENO/–AHR, high FENO/+AHR) are presented in Table 3. More of the patients treated with

inhaled steroid were found to have positive responses to both tests. Patients with atopic disease had no specific distribution of either FENO or AHR to mannitol (Table 3). Those with high FENO and AHR were found to have higher responsiveness than those with AHR and normal FENO (Table 3).

As shown in Table 4, no association was found between asthma severity and the inflammatory variable including FENO and/or AHR to mannitol.

Of the 217 participants, 105 (48%) were non-smokers and were not receiving inhaled corticosteroid (ICS), 44% had ICS prescribed, 5% were smokers, and 3% were current smokers and receiving ICS. No association was found between FENO and use of ICS [23 (24) versus 23 (20), $p=0.7$] or FENO and current smoking [23 (23) versus 15 (8), $p=0.117$], whereas those receiving ICS had more severe airway responsiveness [log RDR 1.8 (0.8) versus 1.4 (0.7), $p<0.001$]. Moreover, the area under the curve (ROC) was almost unchanged when excluding those prescribed ICS (data not shown).

Discussion

The study evaluates FENO and airway hyperresponsiveness to inhaled mannitol in a real-life setting of patients with asthma-like symptoms referred to an asthma clinic, unselected regarding steroid use and tobacco consumption. A substantially weaker association was found between responsiveness to mannitol and FENO than that found in a previous study of selected subjects with asthma [6]. Moreover, our findings showed that 26% had AHR to mannitol without elevated FENO (<25 ppb) and 8% had elevated FENO without AHR to mannitol. More severe airway responsiveness was found in subjects with high FENO compared with those with low FENO, but the present findings were unrelated to the level of asthma control, as shown in Table 4.

A close association between airway inflammation and airway hyperresponsiveness has previously been found in

Table 2. Distribution of response to FENO and AHR to Mannitol in 141 patients who underwent both tests.

		AHR Mannitol		Total
		No (% total)	Yes (% of total)	
NO > 25 ppb	Low	Count	72 (51%)	109 (77%)
	High	Count	11 (8%)	
			21 (15%)	32 (23%)
Total		Count	83 (59%)	141 (100%)
			58 (41%)	

Figure 1. Plot between airway responsiveness (RDR) to mannitol and FENO (ppb) in the entire population ($r=0.32$, $p<0.01$).

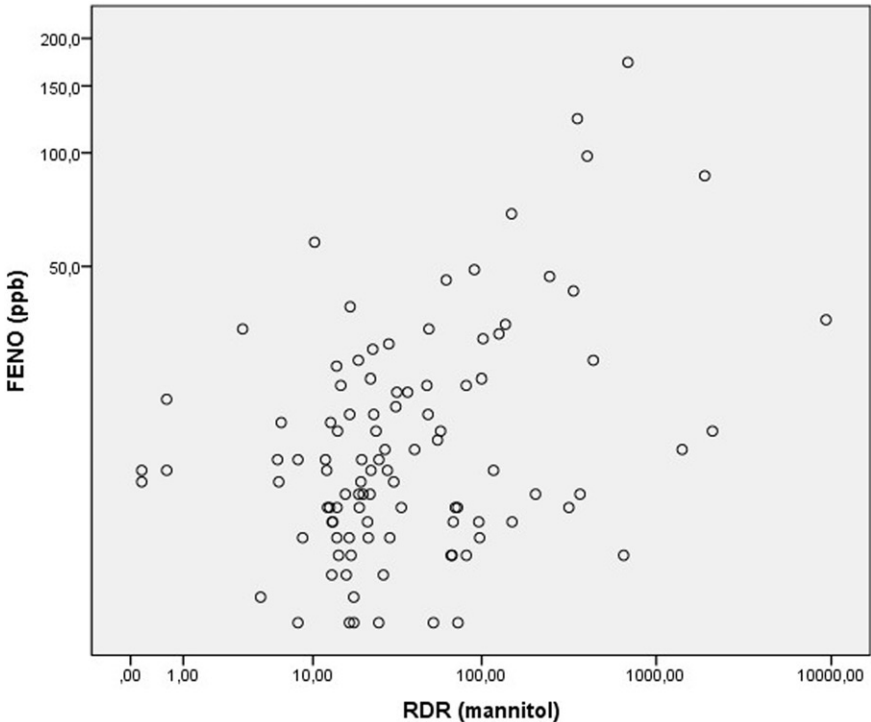


Table 3. Basic characteristics of 141 patients referred to the asthma clinic who had both mannitol and FENO measured (percentage in total).

	Total	Low FENO minus AHR	Low FENO + AHR	High FENO Minus AHR	High FENO + AHR	<i>p</i> Values
N	141	72	37	11	21	
Sex (female)	83 (54%)	45 (54%)	23 (28%)	2 (2%)	13 (16%)	<0.05
Age (years)	28.3 (10)	28.9 (10)	27.0 (10)	28.2 (11)	28.9 (11)	NS
FEV1%	97.2 (16)	97.1 (12)	93.5 (21)	103.4 (16)	100.6 (15)	NS
FVC%	101.8 (16)	101.8 (12)	102.3 (20)	96.0 (17)	103.4 (19)	NS
FEV1/FVC	81 (9)	81 (8)	79 (10)	86 (6)	82 (10)	NS
Resp. symptoms Rest	84.7%	45.1%	19.8%	6.6%	13.2%	NS
Resp. symptoms Activity	85.6%	41.0%	27.3	4.6%	12.5%	NS
Rhinitis and asthma	55	32	14	6	14	<0.001
Asthma	51	20	23	1	7	
Unclear	35	20	0	4	0	
Atopy	127 (90%)	63 (88%)	35 (95%)	11 (100%)	18 (86%)	NS
Inhaled steroid	67 (48%)	30 (42%)	22 (59%)	2 (18%)	13 (61%)	<0.05
Current smokers	4.6%	3.1%	8.6%	9.1%	0.0%	NS
Pack year ^a	7.3 (11)	15.1 (21)	4.5 (3)	0.3 (0)	None	NS
GM RDR mannitol	39.8 (6)	15.8 (2)	123.0 (5) ^c	13.5 (3)	251.2 (4) ^c	<0.001
GM FENO	16.6 (2)	12.0 (2)	13.2 (2)	41.7 (1) ^b	51.3 (2) ^b	<0.001

^aPack year among smokers.

GM: Geometric mean.

Low FENO (<25 ppb), AHR to mannitol (PD15 <635 mg).

^bNon-significant differences.^cSignificant difference, *p* = 0.016.Table 4. Distribution of asthma severity in an unselected group of asthma patients newly referred to an asthma clinic, absolute numbers and % of total in the column in brackets (*p* = 0.2).

GINA	Total (<i>n</i> = 141)	Low FENO and non-AHR (<i>n</i> = 72)	AHR (<i>n</i> = 37)	High FENO (<i>n</i> = 11)	High FENO and AHR (<i>n</i> = 21)
Well controlled	76	41	16	8	11
Partly controlled	10	4	4	1	1
Uncontrolled	55	27	17	2	9

selected groups of asthma patients [6,18–20] and population studies [7,21]. This study, however, represents a heterogeneous sample of subjects with non-specific respiratory symptoms, where some had inhaled steroids prescribed (but with unknown adherence), some might have performed heavy physical activity (cycling) to reach the clinic before testing, and some might have been smokers. These are all factors that influence the results of both FENO and AHR to mannitol, and they are also very commonly seen in the real-life setting outside standardized study protocols. In the tested sample (6% were current smokers, 39% were on ICS and 60% had a positive skin prick test), we found a significant association between response to mannitol and FENO, measured as log RDR to mannitol and log FENO, but the correlation coefficient was low ($r=0.32$), and also significantly lower than in the studies of selected asthma patients and population samples previously mentioned.

Interestingly, a substantial group of subjects (26%) had AHR to mannitol but a FENO below 25 ppb, which differs from earlier findings [10]. The differing results might partly be explained by the inclusion of subjects on ICS and current smokers in this study, unlike the study by Sverrild et al. [10], which is a population survey including both healthy controls and asthma patients. Our findings also indicate that in our group of unselected patients tested in an everyday setting, one test cannot be used to predict

the response to another. Furthermore, as the current patients were examined at their first visit to a specialized asthma clinic, they might represent a group of asthma patients different from those found in the population study by Sverrild et al. [10]. Patients referred to a specialized clinic by a general practitioner (GP) might have difficult-to-treat asthma, and the present results could represent a different phenotype, which with the lack of association between FENO and AHR to mannitol could be explained by a higher number of patients with neutrophilic asthma. AHR to inhaled mannitol is regarded as a marker of steroid responsive airway inflammation, and the findings indicate that (at least some) subjects with low FENO but AHR to mannitol were substantially undertreated. These findings are in agreement with earlier findings of undertreatment in asthma, although those patients had less severe asthma [2].

In general, only minor differences were found between the patients when divided into four groups in terms of high/low FENO and present/absent AHR to mannitol. Only those with AHR but low FENO were found to have more severe airway obstruction compared with the other groups, although not significant. Notably, those were also the patients with the largest proportion of subjects on ICS. Moreover, those with high FENO were the patients with the highest degree of responsiveness, which might explain why these tests used in a real-life setting seem to partly support the

research performed in selected groups and in population studies.

Limitations

The population represents a broad selection of patients with many different respiratory claims, of which some might be asthma, some might not. Additionally, patients might have different anti-asthma drugs prescribed, but whether they were actually taking them is unknown. We posit that this might explain why the level of AHR among those treated with ICS was higher.

The clinical implication of the present findings points towards a specialized asthma clinic having greater diversity than that in former studies by others and by our own research group. Nevertheless, a larger group of patients (51%) had unclear asthma with low FENO and no AHR to mannitol, which might be a group of patients difficult to diagnose. Those difficulties to diagnose in a GP setting should be referred for specialist evaluation. In those patients with low FENO but with AHR, a different phenotype, such as neutrophilic asthma, might be present, although as many as 26% of the patients had this pattern, which is substantially higher than expected. These patients might be the difficult-to-treat asthma patients, who should be followed for a longer period in a specialist setting than those with high FENO and AHR, who would most likely respond fast on ICS due to eosinophilic inflammation. The lack of association between FENO and treatment with inhaled steroid, whereas an association was found between hyperresponsiveness and prescribed ICS, might be due to the word “prescribed medication” in a clinical setting, which might not be the same as therapy taken. In those where the physician meant it to be important to prescribe ICS, they were also those with the most severe AHR. The low adherence among asthma patients might explain the lack of impact on the level of FENO. Overall, although the association between the two tests is less close, the clinical implication of these results could be valuable regarding both the diagnosis of asthma and follow-up after initiation of treatment. Accordingly, more research is needed on the airway inflammation and the distribution of inflammatory cells in the different combination of FENO and AHR found in an out-of-hospital specialized asthma clinic. In selected asthma patients, a close association has been found between response to mannitol and sputum eosinophils as well as high FENO, whereas those with neutrophilic asthma had both low FENO and weaker responsiveness [6]. Lastly, as there is no substantial overlap between the two tests, neither one can be omitted; both are still needed when managing asthma in a real-life setting.

In conclusion, in a large sample of patients with asthma-like symptoms attending a specialized asthma clinic, an association was found between FENO and AHR to mannitol, albeit low. Those patients who had both AHR and high FENO had more severe airway responsiveness than those with low FENO. A significant proportion of patients thought to have asthma had a normal level of FENO despite having AHR, suggesting that in some patients, AHR to mannitol is not driven by eosinophilic airway inflammation, which would have otherwise been expected.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References

1. Thomsen SF, Ulrik CS, Kyvik KO, Larsen K, Skadhaug LR, Steffensen I, Backer V. The incidence of asthma in young adults. *Chest* 2005;127:1928–1934.
2. Nolte H, Nepper-Christensen S, Backer V. Unawareness and undertreatment of asthma and allergic rhinitis in a general population. *Respir Med* 2006;100:354–362.
3. van Weel C, Bateman ED, Bousquet J, Reid J, Grouse L, Schermer T, Valovirta E, Zhong N. Asthma management pocket reference 2008. *Allergy* 2008;63:997–1004.
4. Boulet LP, Fitzgerald JM, Levy ML, Cruz AA, Pedersen S, Haahtela T, Bateman ED. A guide to the translation of the Global Initiative for Asthma (GINA) strategy into improved care. *Eur Respir J* 2012;39:1220–1229.
5. Smith AD, Taylor DR. Is exhaled nitric oxide measurement a useful clinical test in asthma? *Curr Opin Allergy Clin Immunol* 2005;5:49–56.
6. Porsbjerg C, Lund TK, Pedersen L, Backer V. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. *J Asthma* 2009;46:606–612.
7. Sverrild A, Porsbjerg C, Backer V. The use of inhaled mannitol in the diagnosis and management of asthma. *Expert Opin Pharmacother* 2012;13:115–123.
8. Brannan JD, Koskela H, Anderson SD, Chan HK. Budesonide reduces sensitivity and reactivity to inhaled mannitol in asthmatic subjects. *Respirology* 2002;7:37–44.
9. Sverrild A, Porsbjerg C, Thomsen SF, Backer V. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J Allergy Clin Immunol* 2010;126:952–8.
10. Sverrild A, Malinovschi A, Porsbjerg C, Backer V, Alving K. Predicting airway hyperreactivity to mannitol using exhaled nitric oxide in an unselected sample of adolescents and young adults. *Respir Med* 2013;107:150–152.
11. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–615.
12. O’Byrne PM. Global guidelines for asthma management: summary of the current status and future challenges. *Pol Arch Med Wewn* 2010;120:511–517.
13. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143–178.
14. Bousquet J, Schunemann HJ, Zuberbier T, Bachert C, Baena-Cagnani CE, Bousquet PJ, Brozek J, et al. Development and implementation of guidelines in allergic rhinitis – an ARIA-GA2LEN paper. *Allergy* 2010;65:1212–1221.
15. Dreborg S, Belin L, Eriksson NE, Grimmer O, Kunkel G, Malling HJ, Nilsson G, et al. Results of biological standardization with standardized allergen preparations. *Allergy* 1987;42:109–116.
16. Brannan JD, Anderson SD, Perry CP, Freed-Martens R, Lassig AR, Charlton B. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir Res* 2005;6:144.

17. Barnes PJ, Dweik RA, Gelb AF, Gibson PG, George SC, Grasemann H, Pavord ID, et al. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest* 2010;138:682–692.
18. Aronsson D, Tufvesson E, Björner L. Comparison of central and peripheral airway involvement before and during methacholine, mannitol and eucapnic hyperventilation challenges in mild asthmatics. *Clin Respir J* 2011;5:10–18.
19. Sue-Chu M, Brannan JD, Anderson SD, Chew N, Björner L. Airway hyperresponsiveness to methacholine, adenosine 5-monophosphate, mannitol, eucapnic voluntary hyperpnoea and field exercise challenge in elite cross-country skiers. *Br J Sports Med* 2010;44:827–832.
20. Haughney J, Price D, Kaplan A, Chrystyn H, Horne R, May N, Moffat M, et al. Achieving asthma control in practice: understanding the reasons for poor control. *Respir Med* 2008;102:1681–1693.
21. Sverrild A, Porsbjerg C, Thomsen SF, Backer V. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: a random-sample population study. *J Allergy Clin Immunol* 2010;126:952–958.