Allergology International 65 (2016) 439–443

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Association of symptom control with changes in lung function, bronchial hyperresponsiveness, and exhaled nitric oxide after inhaled corticosteroid treatment in children with asthma

Geun-Mi Park [a], Hye Won Han [a], Jae Youn Kim [a], Eun Lee [b], Hyun-Ju Cho [b], Jisun Yoon [b], Soo-Jong Hong [b], Song-I Yang [c], Hyeon-Jong Yang [d], Jinho Yu [b, *]

[a] Department of Pharmacy, Asan Medical Center, Seoul, South Korea
[b] Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea
[c] Department of Pediatrics, Hallym University Sacred Heart Hospital, Anyang, South Korea
[d] Department of Pediatrics, Soonchunhyang University School of Medicine, Seoul, South Korea

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form
14 March 2016
Accepted 30 March 2016
Available online 6 May 2016

Keywords:
Adenosine 5-monophosphate
Asthma
Bronchial hyperresponsiveness
Children
Inhaled corticosteroid

Abbreviations:
ICS, inhaled corticosteroid; BHR, bronchial hyperresponsiveness; eNO, exhaled nitric oxide; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FEF25−75%, forced expiratory flow at 25−75% of forced vital capacity; AMP, adenosine 5-monophosphate; C-ACT, childhood asthma control test; ACQ, asthma control questionnaire

ABSTRACT

Background: A key therapeutic approach to asthma, which is characterized by chronic airway inflammation, is inhaled corticosteroid (ICS). This study evaluated the association of symptom control with changes in lung function, bronchial hyperresponsiveness (BHR), and exhaled nitric oxide (eNO) after ICS treatment in asthmatic children.
Methods: A total of 33 children aged between 5 and 12 years with mild to moderate persistent asthma were treated with 160 μg ciclesonide per day for 3 months. At days 0 and 90, the following parameters were assessed: asthma symptom scores; lung function, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and forced expiratory flow at 25–75% of forced vital capacity ($FEF_{25-75\%}$); BHR to methacholine and adenosine 5-monophosphate (AMP); and eNO.
Results: Asthma symptom scores, lung function parameters, BHR to methacholine and AMP, and eNO levels at day 90 were significantly improved versus day 0 (all $p < 0.001$). Symptom scores at day 90 were not correlated with changes in lung function and BHR to methacholine during the follow-up period, whereas those at day 90 were more closely correlated with changes in BHR to AMP ($r = 0.511$, $p = 0.003$) than with eNO ($r = -0.373$, $p = 0.035$). Additionally, changes in PC_{20} AMP were correlated with changes in PC_{20} methacholine ($r = 0.451$, $p = 0.011$) and eNO ($r = -0.474$, $p = 0.006$).
Conclusions: Changes in the BHR to AMP, and to a lesser extent eNO, correlate with asthma symptom control after ICS treatment. BHR to AMP may better reflect the relationship between improved airway inflammation due to ICS treatment and asthma symptoms.

Introduction

Asthma involves chronic airway inflammation characterized by bronchial hyperresponsiveness (BHR) and reversible airway obstruction. Therefore, treatment with anti-inflammatory agents, such as inhaled corticosteroid (ICS), represents the main effective therapy for asthma management. Current guidelines on asthma management adhere to the concept that treatment should aim to reduce or prevent airway inflammation with ICS and that adjustments of the ICS dose for treatment are guided solely by symptoms and lung function.[1] However, the current stepwise strategy for symptom and lung function optimization does not lead to proper control of asthma in all patients.[2] Moreover, in patients with asthma that is considered to be under control, airway inflammation can persist[3–5] and such abnormalities can cause airway remodeling and reductions in lung function during the long-term follow-up period.[6] Therefore, objective evaluation of airway inflammation and its consequences, as well as the evaluation of symptoms, is needed to achieve proper asthma control.

* Corresponding author. Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 138-736, South Korea.
E-mail address: jyu3922@gmail.com (J. Yu).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.03.011

Many studies have tried to use BHR or inflammation markers as objective markers of the effects of ICS.[2,7,8] It is generally accepted that airway inflammation contributes to the presence and severity of BHR, but reports of a direct association between airway inflammation and BHR have varied according to the method used to measure BHR.[5,9,10] Several studies have shown that BHR to adenosine 5-monophosphate (AMP), an indirect stimulus, is an earlier and more sensitive indicator of the effects of ICS than BHR to methacholine, a direct stimulus.[11–14] In addition, exhaled nitric oxide (eNO) reflects airway inflammation, and changes in eNO after ICS are rapid and reproducible,[15–17] although eNO levels seem to be affected by several factors.[18–22]

However, there is little information to simultaneously relate these objective makers, including BHR to direct or indirect stimuli and airway inflammation markers, to asthma symptom control during ICS treatment in children with asthma. Accordingly, the present study aimed to evaluate the association of symptom scores with changes in lung function, BHR to methacholine or AMP, and eNO after ICS treatment in children with asthma.

Methods

Subjects and study design

A series of 33 children with mild to moderate persistent asthma, aged 5–12 years, was recruited from August to October 2012 from the Childhood Asthma Atopy Center at Asan Medical Center Children's Hospital. All subjects met the following criteria: airway reversibility to β_2-agonist $\geq$12% of the predicted forced expiratory volume in one second (FEV_1) and/or symptom relief using a bronchodilator, a history of recurrent wheezing and/or dyspnea within the previous 12 months, and no severe comorbidities, including bronchiolitis obliterans, malignancy, and congenital heart disease affecting lung function. Before treatment with ICS, all patients were (by design) responsive to methacholine (provocative concentration causing a 20% fall in FEV_1, $PC_{20} \leq 25$ mg/mL) and AMP ($PC_{20} \leq 400$ mg/mL). Definition of disease severity was based on the criteria set in the National Asthma Education and Prevention Program (NAEPP) guidelines.[1]

All subjects were treated with 160-μg ciclesonide per day (Alvesco®, Takeda Pharmaceuticals, Dubendorf, Switzerland) for 3 months, which was administrated with or without a spacer (Vortex®, PARI GmbH, Starnberg, Germany) that was fitted to the mouthpiece, depending on the patient age and inhaler performance. At both days 0 and 90, the following parameters were assessed: asthma symptom scores; lung function, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and forced expiratory flow at 25%–75% of forced vital capacity ($FEF_{25-75\%}$); BHR to methacholine and AMP; and levels of eNO.

The study protocol was approved by the Institutional Review Board of the Asan Medical Center and all participants gave written informed consent after receiving a detailed explanation of the study.

Measurements of lung function and BHR

Basal lung function, including measurements of the FEV_1, FVC, and $FEF_{25-75\%}$, and two bronchial provocation test with methacholine and AMP were performed in all subjects on the same day. The value of the FEV_1 and $FEF_{25-75\%}$ were expressed as a percentage of the predicted value for the global lung function 2012 equations.[23] After methacholine challenge, an AMP challenge was carried out after recovery of the FEV_1 to within 5% of the baseline FEV_1 of a methacholine challenge. Antihistamines, bronchodilators, and other medications were not taken for 48 h before testing on days 0 and 90. ICS administration was stopped for 14 days before testing at day 0, but was continued at day 90.

Methacholine and AMP were prepared in 0.9% saline solution at concentrations of 0.625–25 mg/mL for methacholine (0.625, 1.25, 2.5, 5, 10, and 25 mg/mL) and 3.125–400 mg/mL for AMP (3.125, 6.25, 12.5, 50, 100, 200, and 400 mg/mL). The FVC and FEV_1 values were measured at 1 and 3 min in the methacholine and AMP tests after each administration. The challenge was terminated if FEV_1 dropped by >20% from post-saline value or if maximal concentration of methacholine or AMP was administered. PC_{20} was calculated by linear interpolation of the log-dose-response curves.

Measurements of eNO levels

The eNO fraction was measured using a Niox Mino device (Aerocrine, Solna, Sweden) using a previously described method before bronchial provocation test.[24]

Asthma control assessments

We modified a previous questionnaire for assessing asthma symptom scores.[25,26] Patients were asked to recall their symptoms during the previous month at each visit, and symptom scores included wheezing, use of a short-acting bronchodilator, shortness of breath, nocturnal symptoms, activity limitation, and overall asthma control. All six questions were scored on a 5-point scale, and a high score indicated good asthma control.

Measurements of atopy

A skin prick test (SPT) was performed with 31 common allergens using standard methods[27]: *Dermatophagoides pteronyssinus*, *D. farinae*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, grasses, trees, weeds, ragweed, mugwort, oak, beech, nettle, willow, elm, pine, hop, elder, hazel, oats, lambs quarter, ash, alder, birch, timothy, rye grass, dog, cat, and cockroach. SPT was included a positive control (histamine) and a negative control (isotonic saline). A positive on SPT was defined as a mean wheal diameter of $\geq$3 mm and greater than that of the histamine. Atopy was defined as positive SPT result to at least one allergen.

Total serum IgE and blood eosinophils

Total serum IgE levels were measured with immunoCAP system (Phadia AB, Uppsala, Sweden). Blood eosinophil counts were measured using an automated blood analyzer.

Statistical analyses

Data are presented as means ± SD or as geometric means with a range of 1 SD. Levels of total IgE, blood eosinophil counts, PC_{20} methacholine, PC_{20} AMP, and eNO were log-transformed prior to analysis to normalize the distribution or these values. Variables were then compared using the paired *t*-test, and frequencies were compared using the χ^2 test. Correlations between variables were analyzed using Pearson's correlation test. Changes in PC_{20} methacholine and PC_{20} AMP after 3 months of ICS treatment versus pretreatment values were expressed as dose shifts (in doubling doses) using the following formula: $\Delta\log_{10}PC_{20} = [\log_{10}(PC_{20}$ after the treatment) $- \log_{10}(PC_{20}$ before the treatment)]/$\log_{10}2$.[28,29] A p-value of 0.05 or less was considered to be significant. The SPSS version 19 software package was used for these analyses (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study subjects

The mean age of the 33 subjects was 6.95 ± 1.83 years, and the proportions of male patients and atopy were 72.7% and 81.8%, respectively. The FEV_1, $FEF_{25-75\%}$, and FEV_1/FVC values were 99.06 ± 10.92% pred (predicted), 89.45 ± 26.57% pred, and 0.86 ± 0.09, respectively. The geometric means (range of 1SD) of PC_{20} methacholine, PC_{20} AMP, eNO, blood eosinophils, and total IgE were 2.82 (1.20–6.61), 63.10 (17.78–223.87), 19.50 (10.96–34.67), 5.25 (2.82–9.77), and 257.04 (83.18–794.33), respectively. The characteristics of the children analyzed in this study are listed in Table 1.

Changes in lung function, BHR, eNO, and symptom scores after 3 months of ICS treatment

We examined the symptom scores, lung function, BHR, and eNO at days 0 and 90 (Table 2). The asthma symptom scores, parameters for lung function, BHR to methacholine and AMP, and levels of eNO at day 90 were all significantly improved compared with those at day 0 (all $p < 0.001$). The changes in asthma symptom scores, FEV_1% pred, and $FEF_{25-75\%}$% pred after 3 months of ICS treatment were 3.36 ± 4.39, 12.21 ± 12.47, and 20.88 ± 32.00, respectively. The changes in PC_{20} methacholine and PC_{20} AMP, expressed as doubling doses, were 2.16 ± 1.54 and 2.91 ± 1.59 after 3 months of ICS treatment, respectively. The change in the log eNO values was −0.28 ± 0.33 after 3 months of ICS treatment.

The rates of BHR to methacholine (<8 mg/mL) were 87.9% at day 0 and 45.5% at day 90. The rates of BHR to AMP (<200 mg/mL) were 84.8% at day 0 and 15.2% at day 90. The rates of positive responses in eNO (>20 ppb in subjects younger than 12 years and >25 ppb in subjects older than 12 years) were 54.5% at day 0 and 21.2% at day 90. The rates of BHR to methacholine or AMP and positive responses in eNO were significantly reduced after 3 months of ICS treatment (all $p < 0.05$).

Associations between symptom scores at day 90 and changes in lung function, BHR, and levels of eNO

At baseline, symptom scores were not correlated with lung function, BHR to methacholine or AMP, or eNO levels. However, the levels of FEV_1% pred were correlated with the levels of PC_{20} methacholine, PC_{20} AMP, and eNO ($r = 0.476$, $p < 0.01$; $r = 0.416$, $p < 0.05$; and $r = -0.384$, $p < 0.05$, respectively) at day 0, and the levels of PC_{20} AMP were correlated with the levels of eNO at day 0 ($r = -0.467$, $p < 0.01$; Table 3). Symptom scores at day 90 were not correlated with changes in lung function or PC_{20} methacholine after 3 months of treatment (Table 4). However, the symptom scores at day 90 were correlated with the changes in PC_{20} AMP ($r = 0.511$, $p < 0.01$) and log eNO ($r = -0.373$, $p < 0.05$) after 3 months of treatment. Additionally, the changes in PC_{20} AMP were correlated with the changes in PC_{20} methacholine ($r = 0.451$, $p < 0.05$) and log eNO ($r = -0.474$, $p < 0.01$) after 3 months of treatment.

Table 1
Baseline characteristics of the study subjects.

Variables	N = 33
Age, years	6.95 ± 1.83
Males/females	24/9
Height, cm	120.48 ± 11.59
Weight, kg	24.19 ± 9.55
Atopy, n (%)	27 (81.8%)
Allergic rhinitis	21 (63.6%)
Symptom score	24.42 ± 4.38
FEV_1% pred	99.06 ± 10.92
FEV_1/FVC	0.86 ± 0.09
$FEF_{25-75\%}$% pred	89.45 ± 26.57
PC_{20} methacholine, mg/mL	2.82 (1.20–6.61)
PC_{20} AMP, mg/mL	63.10 (17.78–223.87)
Exhaled nitric oxide, ppb	19.50 (10.96–34.67)
Blood eosinophils/mm^3 (%)	5.25 (2.82–9.77)
Total IgE, KU/L	257.04 (83.18–794.33)
Asthma severity, n (%)	
Mild, intermittent	0
Mild, persistent	30 (90.9%)
Moderate, persistent	3 (9.1%)
Severe, persistent	0

Data are expressed as means ± SD or geometric means (range of 1 SD).

Table 2
Changes in symptom scores, lung function, bronchial hyperresponsiveness, and exhaled NO during the follow-up period.

	Day 0	Day 90	Change from baseline
Symptom score	24.42 ± 4.38	27.79 ± 2.67*	3.36 ± 4.39
FEV_1% pred	99.06 ± 10.92	110.56 ± 12.77*	12.21 ± 12.47
$FEF_{25-75\%}$ pred	89.45 ± 26.57	105.07 ± 31.07*	20.88 ± 32.00
PC_{20} methacholine, mg/mL†	2.82 (1.20–6.61)	12.59 (3.31–47.86)*	2.16 ± 1.54
PC_{20} AMP, mg/mL†	63.10 (17.78–223.87)	478.63 (141.25–1621.81)*	2.91 ± 1.59
eNO, ppb‡	19.50 (10.96–34.67)	10.23 (4.90–21.38)*	−0.28 ± 0.33

Data are expressed as means ± SD or geometric means (range of 1 SD).
† Changes in PC20 methacholine and PC20 AMP versus pretreatment values were expressed as doubling doses.
‡ Changes in log eNO versus pretreatment values.
* $p < 0.001$.

Discussion

In our present study, asthma symptom scores and the parameters for lung function, BHR, and levels of eNO improved significantly after 3 months of ICS treatment. Although changes in lung function and BHR to methacholine during ICS treatment were not correlated with asthma symptom scores, changes in BHR to AMP and levels of eNO were correlated with asthma symptom scores. Furthermore, changes in the BHR to AMP showed a more robust correlation with asthma symptom scores than changes in the levels of eNO.

In our current analyses, asthma control based on symptom scores was found to be associated with changes in BHR to AMP after ICS treatment but was not correlated with changes in BHR to methacholine. Methacholine, a direct stimulus, acts directly on the airway smooth muscle causing the airway obstruction in patients with asthma.[30,31] Measurements of BHR to methacholine are more closely associated with FEV_1, which represents baseline airway caliber, than BHR to AMP, an indirect stimulus, which represents a better indicator of airway inflammation in asthmatic

Table 3
Relationships between the symptom score, lung function, BHR, and eNO at day 0.

		FEV_1% pred	$FEF_{25-75\%}$ pred	log PC_{20} methacholine	log PC_{20} AMP	log eNO
Symptom score	r	0.158	0.107	0.152	0.001	0.076
FEV_1% pred	r	–	0.538*	0.476*	0.416**	−0.384**
$FEF_{25-75\%}$ pred	r	–	–	0.491*	0.360**	−0.288
log PC_{20} methacholine	r	–	–	–	0.191	−0.144
log PC_{20} AMP	r	–	–	–	–	−0.467*

* $p < 0.01$.
** $p < 0.05$.

Table 4
Relationships between symptom scores and changes in lung function, BHR, and eNO at day 90.

		ΔFEV_1% pred	$\Delta FEF_{25-75\%}$% pred	ΔPC_{20} methacholine†	ΔPC_{20} AMP†	ΔeNO‡
Symptom score	r	−0.088	−0.110	0.278	0.511*	−0.373**
ΔFEV_1% pred	r	–	0.717*	0.311	0.319	0.159
$\Delta FEF_{25-75\%}$% pred	r	–	–	0.289	0.272	−0.089
ΔPC_{20} methacholine	r	–	–	–	0.451**	−0.099
ΔPC_{20} AMP	r	–	–	–	–	−0.474*

† Changes in PC_{20} methacholine and PC_{20} AMP versus pretreatment values were expressed as doubling doses.
‡ Changes in log eNO versus pretreatment values.
* $p < 0.01$.
** $p < 0.05$.

patients.[11,32,33] In contrast to the direct effect of methacholine on smooth muscle, AMP acts on mast cells by binding to the adenosine A2B receptor and increasing the release of inflammatory mediators such as histamine, prostaglandins, tryptase, and leukotrienes from mast cells.[34,35] Additionally, other studies demonstrated that PC_{20} AMP is a more sensitive method for evaluating airway inflammation after ICS treatment than PC_{20} methacholine.[12,36] These previous results support our finding that asthma symptom scores are associated with changes in BHR to AMP rather than changes in BHR to methacholine after ICS treatment.

Levels of eNO are related to eosinophilic airway inflammation, measured in induced sputum and bronchoalveolar lavage fluid,[15,16] and the decrease in the levels of eNO during ICS treatment is rapid and reproducible.[17] In the present study, asthma symptom scores were also associated with changes in the levels of eNO. Notably, however, asthma symptom scores were found to be more closely correlated with changes in BHR to AMP than changes in the levels of eNO after ICS treatment. Moreover, changes in BHR to methacholine were correlated with changes in BHR to AMP after ICS treatment but not with changes in the levels of eNO. BHR to AMP seems to have a component of BHR in response to ICS beyond airway inflammation, represented by eNO levels. Only 60.7% of our study subjects with BHR to AMP (<200 mg/mL) had higher levels of eNO (>20 ppb in subjects younger than 12 years and >25 ppb in subjects older than 12 years). The mechanism of mast cell mediator release induced by challenge with mannitol, another indirect stimulus, is similar to that of AMP challenge and causes bronchoconstriction.[37] Almost 20% of asthmatic patients who had BHR to mannitol had normal levels of eNO.[38] In addition, BHR to mannitol was not significantly different between eosinophilic and non-eosinophilic asthma phenotypes, whereas the levels eNO were significantly different between those phenotypes.[39]

The recent addition of eNO measurements to the present guidelines for asthma management has resulted in the administration of higher doses of ICS but has not achieved clinically significant improvements in asthma control.[40] Moreover, eNO levels are affected by several factors, including atopy, total IgE, other allergic diseases, exposure to allergens, height, and food.[18–22] Therefore, measurement of BHR to AMP might be a better tool to predict the response to ICS than eNO levels, although conducting a provocation test with AMP can be time-consuming.

In the current guidelines, the evaluation of asthma symptoms that are subjectively reported by asthmatic children and their parents is a core asthma outcome measurement. However, many children with asthma are poor perceivers of airway obstruction and vary considerably in the degree of airway narrowing that they recognize.[41,42] Psychological factors are highly associated with asthma symptom burden[43] and increased requests for asthma medication.[44] Considering these demerits of asthma symptoms, treatment based on symptoms alone may lead to either overtreatment or undertreatment. Moreover, FEV_1, an objective measurement included in the current guidelines, does not correlate well with the magnitude of asthma symptoms in children.[45] Therefore, objective measurements of the effect of ICS as well as the evaluation of asthma symptoms are needed to achieve better asthma control.

Our current study is the first to evaluate the associations of asthma symptom scores with changes in lung function, BHR to methacholine or AMP, and levels of eNO after ICS treatment in children with asthma. However, our study period was too short to make any strong inferences regarding the clinical application of monitoring tools for asthma control. The childhood asthma control test (C-ACT)[26] and the asthma control questionnaire (ACQ)[25] have been extensively used to evaluate asthma symptoms in children with asthma. These two instruments consist of similar questions except for a question on the use of rescue medication, which is included in the ACQ but not in the C-ACT. We believe that use of rescue medication is an important factor for the assessment of asthma control and have thus modified the C-ACT. We validated the questionnaire used in this study, but the validation results have unfortunately not been published yet (data not shown). All subjects stopped ICS treatment for 14 days before testing at day 0. Two weeks might be too short a period to neutralize the effects of ICS on outcome values at day 0. However, all outcome values for the comparison were assessed on the same day in each individual at day 0 under the same conditions of a 2-week ICS withdrawal. There is still a limitation that ICS might affect some outcome values, particularly BHR to AMP and eNO, at day 0 and thus affect the change in these values between day 0 and day 90 more than other values, such as lung function.

In addition, we did not measure adherence to ICS during our current study period. However, each value was assessed in each individual under the same conditions of adherence. Most of our subjects had mild persistent asthma, so our results might not be representative of the entire asthmatic population. Although the degree of change in each value was not high because the subjects had mild persistent asthma, we observed significant relationships between changes in each value and asthma symptoms. Additionally, AMP challenge was performed after methacholine challenge on the same day. It is unlikely that methacholine challenge influenced the AMP challenge results because we performed the AMP challenge when the baseline FEV_1 had recovered to within 5% of the baseline for methacholine challenge without any respiratory symptoms that suggested airway obstruction. Therefore, baseline airway calibers after methacholine challenge were not likely to affect the PC_{20} for AMP challenge. Actually, in our present study, the baseline FEV_1 values for the two challenges were not significantly different (data not shown).

In conclusion, improvements in BHR to AMP and levels of eNO after ICS treatment show a correlation with asthma symptom scores, whereas improvements in lung function and BHR to methacholine do not. Furthermore, BHR to AMP exhibits a more robust correlation with symptom scores than the levels of eNO. BHR to AMP may better reflect the relationship between the improvement in airway inflammation by treatment with ICS and asthma symptoms. These findings suggest that PC_{20} AMP may be an important parameter for the management of children with asthma receiving ICS. Further long-term studies designed to assess whether the use of PC_{20} AMP as a monitoring tool for asthma can improve asthma control in the clinic are needed.

Acknowledgments

The authors would like to thank all of the study participants and their parents. This study was supported by a grant (2015-644) from

the Asan Institute for Life Sciences, Asan Medical Center, Seoul, Korea.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

GP performed research, analyzed data, and wrote the paper. HH and JK supervised GP's work. JY and HY contributed to the data analysis and the preparation and revision of the manuscript. Other authors treated asthma patients who participated in the study.

References

1. Urbano FL. Review of the NAEPP 2007 Expert Panel Report (EPR-3) on asthma diagnosis and treatment guidelines. *J Manag Care Pharm* 2008;**14**:41–9.
2. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. *Am J Respir Crit Care Med* 1999;**159**:1043–51.
3. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;**157**:4–9.
4. Boulet LP, Turcotte H, Brochu A. Persistence of airway obstruction and hyperresponsiveness in subjects with asthma remission. *Chest* 1994;**105**:1024–31.
5. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, et al. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;**51**:496–502.
6. Lange P, Parner J, Vestbo J, Schnohr P, Jensen GA. 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;**339**:1194–200.
7. Nuijsink M, Hop WC, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. *Eur Respir J* 2007;**30**:457–66.
8. Petsky HL, Kynaston JA, Turner C, Li AM, Cates CJ, Lasserson TJ, et al. Tailored interventions based on sputum eosinophils versus clinical symptoms for asthma in children and adults. *Cochrane Database Syst Rev* 2007;**18**:CD005603.
9. Jatakanon A, Kharitonov S, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. *Thorax* 1999;**54**:108–14.
10. Leuppi JD, Salome CM, Jenkins CR, Koskela H, Brannan JD, Anderson SD, et al. Markers of airway inflammation and airway hyperresponsiveness in patients with well-controlled asthma. *Eur Respir J* 2001;**18**:444–50.
11. van den Berge M, Kerstjens HA, Meijer RJ, de Reus DM, Koeter GH, Kauffman HF, et al. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;**164**:1127–32.
12. Weersink EJ, Douma RR, Postma DS, Koeter GH. Fluticasone propionate, salmeterol xinafoate, and their combination in the treatment of nocturnal asthma. *Am J Respir Crit Care Med* 1997;**155**:1241–6.
13. Kerstjens HA, Brand PL, Hughes MD, Robinson NJ, Postma DS, Sluiter HJ, et al. A comparison of bronchodilator therapy with or without inhaled corticosteroid therapy for obstructive airways disease. Dutch Chronic Non-Specific Lung Disease Study Group. *N Engl J Med* 1992;**327**:1413–9.
14. Prosperini G, Rajakulasingam K, Cacciola RR, Spicuzza L, Rorke S, Holgate ST, et al. Changes in sputum counts and airway hyperresponsiveness after budesonide: monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. *J Allergy Clin Immunol* 2002;**110**:855–61.
15. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000;**106**:638–44.
16. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JD, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;**57**:383–7.
17. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001;**119**:1322–8.
18. Woo SI, Lee JH, Kim H, Kang JW, Sun YH, Hahn YS. Utility of fractional exhaled nitric oxide (F(E)NO) measurements in diagnosing asthma. *Respir Med* 2012;**106**:1103–9.
19. Janson C, Kalm-Stephens P, Foucard T, Norback D, Alving K, Nordvall SL. Exhaled nitric oxide levels in school children in relation to IgE sensitisation and window pane condensation. *Respir Med* 2005;**99**:1015–21.
20. Ihre E, Gyllfors P, Gustafsson LE, Kumlin M, Dahlen B. Early rise in exhaled nitric oxide and mast cell activation in repeated low-dose allergen challenge. *Eur Respir J* 2006;**27**:1152–9.
21. Vints AM, Oostveen E, Eeckhaut G, Smolders M, De Backer WA. Time-dependent effect of nitrate-rich meals on exhaled nitric oxide in healthy subjects. *Chest* 2005;**128**:2465–70.
22. Malmberg LP, Petays T, Haahtela T, Laatikainen T, Jousilahti P, Vartiainen E, et al. Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values. *Pediatr Pulmonol* 2006;**41**:635–42.
23. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;**40**:1324–43.
24. Shim E, Lee E, Yang SI, Jung YH, Park GM, Kim HY, et al. The association of lung function, bronchial hyperresponsiveness, and exhaled nitric oxide differs between atopic and non-atopic asthma in children. *Allergy Asthma Immunol Res* 2015;**7**:339–45.
25. Juniper EF, Gruffydd-Jones K, Ward S, Svensson K. Asthma control questionnaire in children: validation, measurement properties, interpretation. *Eur Respir J* 2010;**36**:1410–6.
26. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the childhood asthma control test. *J Allergy Clin Immunol* 2007;**119**:817–25.
27. Heinzerling L, Frew AJ, Bindslev-Jensen C, Bonini S, Bousquet J, Bresciani M, et al. Standard skin prick testing and sensitization to inhalant allergens across Europe – a survey from the GALEN network. *Allergy* 2005;**60**:1287–300.
28. Cockcroft DW, McParland CP, Britto SA, Swystun VA, Rutherford BC. Regular inhaled salbutamol and airway responsiveness to allergen. *Lancet* 1993;**342**:833–7.
29. Koh YY, Lee MH, Sun YH, Park Y, Kim CK. Improvement in bronchial hyperresponsiveness with inhaled corticosteroids in children with asthma: importance of family history of bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2002;**166**:340–5.
30. Van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. *Eur Respir J* 2000;**16**:514–33.
31. Cockcroft DW. Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;**138**:18S–24.
32. De Meer G, Heederik D, Postma DS. Bronchial responsiveness to adenosine 5′-monophosphate (AMP) and methacholine differ in their relationship with airway allergy and baseline FEV(1). *Am J Respir Crit Care Med* 2002;**165**:327–31.
33. Van Den Berge M, Meijer RJ, Kerstjens HA, de Reus DM, Koeter GH, Kauffman HF, et al. PC(20) adenosine 5′-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. *Am J Respir Crit Care Med* 2001;**163**:1546–50.
34. Feoktistov I, Biaggioni I. Adenosine A2b receptors evoke interleukin-8 secretion in human mast cells. An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* 1995;**96**:1979–86.
35. van den Berge M, Polosa R, Kerstjens HA, Postma DS. The role of endogenous and exogenous AMP in asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2004;**114**:737–46.
36. Taylor DA, Jensen MW, Kanabar V, Engelstatter R, Steinijans VW, Barnes PJ, et al. A dose-dependent effect of the novel inhaled corticosteroid ciclesonide on airway responsiveness to adenosine-5′-monophosphate in asthmatic patients. *Am J Respir Crit Care Med* 1999;**160**:237–43.
37. Brannan JD, Gulliksson M, Anderson SD, Chew N, Kumlin M. Evidence of mast cell activation and leukotriene release after mannitol inhalation. *Eur Respir J* 2003;**22**:491–6.
38. Porsbjerg C, Brannan JD, Anderson SD, Backer V. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. *Clin Exp Allergy* 2008;**38**:43–50.
39. 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**:606–12.
40. Szefler SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, et al. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet* 2008;**372**:1065–72.
41. Brouwer AF, Brand PL, Roorda RJ, Duiverman EJ. Airway obstruction at time of symptoms prompting use of reliever therapy in children with asthma. *Acta Paediatr* 2010;**99**:871–6.
42. Horak E, Grassl G, Skladal D, Ulmer H. Lung function and symptom perception in children with asthma and their parents. *Pediatr Pulmonol* 2003;**35**:23–8.
43. Richardson LP, Lozano P, Russo J, McCauley E, Bush T, Katon W. Asthma symptom burden: relationship to asthma severity and anxiety and depression symptoms. *Pediatrics* 2006;**118**:1042–51.
44. Dahlem NW, Kinsman RA, Horton DJ. Panic-fear in asthma: requests for as-needed medications in relation to pulmonary function measurements. *J Allergy Clin Immunol* 1977;**60**:295–300.
45. Sharek PJ, Mayer ML, Loewy L, Robinson TN, Shames RS, Umetsu DT, et al. Agreement among measures of asthma status: a prospective study of low-income children with moderate to severe asthma. *Pediatrics* 2002;**110**:797–804.