



The Association of Lung Function, Bronchial Hyperresponsiveness, and Exhaled Nitric Oxide Differs Between Atopic and Non-atopic Asthma in Children

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Purpose: Although many previous studies have attempted to identify differences between atopic asthma (AA) and non-atopic asthma (NAA), they have mainly focused on the difference of each variable of lung function and airway inflammation. The aim of this study was to evaluate relationships between lung function, bronchial hyperresponsiveness (BHR), and the exhaled nitric oxide (eNO) levels in children with AA and NAA.

Methods: One hundred and thirty six asthmatic children aged 5-15 years and 40 normal controls were recruited. Asthma cases were classified as AA (n=100) or NAA (n=36) from skin prick test results. Lung function, BHR to methacholine and adenosine-5'-monophosphate (AMP), eNO, blood eosinophils, and serum total IgE were measured. **Results:** The AA and NAA cases shared common features including a reduced small airway function and increased BHR to methacholine. However, children with AA showed higher BHR to AMP and eNO levels than those with NAA. When the relationships among these variables in the AA and NAA cases were evaluated, the AA group showed significant relationships between lung function, BHR to AMP or methacholine and eNO levels. However, the children in the NAA group showed an association between small airway function and BHR to methacholine only. **Conclusions:** These findings suggest that the pathogenesis of NAA may differ from that of AA during childhood in terms of the relationship between lung function, airway inflammation and BHR.

Key Words: Asthma; atopy; child; lung function, bronchial hyperresponsiveness; exhaled nitric oxide

INTRODUCTION

Asthma has generally been defined as a chronic airway inflammatory disorder characterized by bronchial hyperresponsiveness (BHR) and recurrent airflow obstruction. However, asthma is a heterogeneous condition with distinct phenotypes and endotypes.¹ In addition, wheezing in childhood is a heterogeneous condition, and 2 main phenotypes are atopic and non-atopic after the age of 6 years.^{2,3} Although a large majority of non-atopic children with wheeze spontaneously resolves in later childhood, atopic children with wheeze are more likely to develop chronic asthma.⁴ This suggests that children with non-atopic asthma (NAA) would be predicted to have a different pathophysiology from those with atopic asthma (AA) during childhood.

Clinical severity and lung function have been reported to be similar in both AA and NAA children.⁵⁻⁷ In addition, no difference was reported in the airway pathologic features of eosino-

philic inflammation and remodeling in bronchial biopsies between children with AA and NAA.⁸ However, the exhaled nitric oxide (eNO) levels and BHR, especially to adenosine-5'-monophosphate (AMP), were found to be greater in AA.^{7,9} Moreover, the proportion of eosinophils in induced sputum is higher in children with AA.^{10,11}

Bronchoprovocation challenge has been widely used for measuring BHR in patients with asthma, and stimuli used can be categorized as direct stimuli, such as methacholine, or indirect stimuli, such as AMP.¹² BHR to methacholine is more closely as-

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sociated with baseline forced expiratory volume in 1 sec (FEV1), whereas BHR to AMP is a better marker of airway inflammation in asthmatics.^{13,14} The eNO has been identified as a non-invasive marker of airway inflammation¹⁵ and found to have a significant correlation with BHR to AMP in adults with asthma.¹⁶ However, no previous study has compared the relationships between lung function, BHR to direct and indirect stimuli, and eNO as a possible way to differentiate AA from NAA in children.

The aims of this study were to compare lung function, BHR, and the eNO levels in children with AA and NAA and to then evaluate the relationship among these factors in each patient group to clarify any differences in the pathophysiology between AA and NAA.

MATERIALS AND METHODS

Subjects

One hundred and thirty-six children with asthma, aged 5-15 years, were recruited from October 2010 to May 2012 from the Childhood Asthma, Atopy Center at the Asan Medical Center Children's Hospital. All subjects had a history of recurrent wheezing and/or dyspnea within previous 12 months, and had been diagnosed on the basis of airway reversibility defined by an increased FEV1 of $\geq 12\%$ after bronchodilator administration and/or symptom relief using a bronchodilator. Patients with severe comorbidities, including congenital heart disease, bronchiolitis obliterans, and malignancy, affecting lung function were excluded. Asthma severity was defined according to the National Asthma Education and Prevention Program (NAEPP) guidelines.¹⁷ Asthmatic children were classified as AA (n=100) or NAA (n=36) from skin prick test (SPT) results.

A non-atopic control (NAC) group of 40 children who visited our hospital over the same period for routine examinations and had no allergic diseases and a negative SPT were also recruited. Our study protocol was approved by the Institutional Review Board of Asan Medical Center, and all parents and guardians provided written informed consent following a detailed explanation of the study.

Measurement of lung function and BHR

Basal lung function, including measurements of FEV1, forced vital capacity (FVC), and forced expiratory flow at 25% to 75% of FVC (FEF_{25%-75%}) was assessed prior to methacholine challenge; percentage predicted values (% pred) for FEV1, FVC, and FEF_{25%-75%} were calculated based on previously determined reference values for healthy Korean children.¹⁸ The bronchial provocation test with methacholine followed by AMP was performed in all subjects on the same day. An AMP challenge was performed after recovery of FEV1 to within 5% of the baseline FEV1 of a methacholine challenge. All subjects were requested to stop using antihistamines, bronchodilators, and other medi-

cations for 48 hours and inhaled corticosteroids for 14 days before testing. None of our subjects had any symptoms of upper respiratory tract infections in the month preceding the tests.

Fresh preparations of methacholine and AMP were made in buffered saline solution at concentrations of 0.625 to 25 mg/mL for methacholine (0.625, 1.25, 2.5, 5, 10, and 25 mg/mL) and 3.125 to 400 mg/mL for AMP (3.125, 6.25, 12.5, 50, 100, 200, and 400 mg/mL). FVC and FEV1 values were determined at each time point in methacholine and AMP tests when there was a concentration change. A dose-response slope (DRS) was calculated to overcome the information limit arising from the censored value given to children with low BHR to methacholine or AMP. The slope was estimated by dividing the percentage fall from the post-saline FEV1 at the highest dose given by that final dose, and a constant was added to remove negative values that can occur in subjects with low BHR.¹⁹ These values are then log transformed to normalize the distribution. The formula we used is as follows:

$$\text{DRS} = \text{Log}_{10} \left[\frac{(\text{post-saline FEV1}) - (\text{final dose FEV1})}{(\text{post-saline FEV1}) \times 100 / (\text{cumulative dose of methacholine or AMP}) + 3.2} \right]$$

Measurement of eNO

The eNO fraction was measured using a Niox Mino device (Aerocrine, Solna, Sweden). The measurements were performed at an expiratory flow rate of 50 mL/sec, and the duration of exhalation was at least 6 seconds to ensure a stable NO level. Three measurements were obtained for each subject, and the mean value was recorded as the level of eNO.

Measurement of atopy

The SPT was performed on the backs of the children using standard methods.²⁰ Commercial extracts of the following common allergens were used: mites (*D. pteronyssinus* and *D. farina*), molds (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*), pollens (grasses, trees, weeds, ragweed, mugwort, oak, beech, nettle, willow, elm, pine, hop, elder, hazel, oats, lambs quarter, ash, alder, birch, timothy, and rye grass), animal dander (dog, and cat), and cockroach. Histamine and isotonic saline were used as positive and negative controls. A positive SPT was defined as a mean wheal diameter of ≥ 3 mm and greater than that of the positive control. Atopy was defined as the presence of at least 1 positive SPT result. In addition, the degree of atopy was measured by sum of wheal diameters of positive allergens tested.

Blood eosinophils and serum total IgE

Blood eosinophil counts were measured using an automated blood analyzer. Serum total IgE levels were measured with a fluorescence enzyme immunoassay using the UniCAP system (ThermoFisher Scientific Inc., Uppsala, Sweden).

Statistical analysis

Data are presented as the mean \pm SD or as the geometric mean with a range of 1 SD. The levels of total IgE, blood eosinophil counts, and sum of wheals, and DRS AMP and methacholine were log-transformed before analysis to normalize their distributions. The variables were then compared using Student's *t* test or analysis of variance (ANOVA), and the frequencies were compared using the χ^2 test. Correlations between variables were analyzed using Pearson's correlation test with controls for sex and height as they affect each of these variables in bivariate correlation analysis. A *P* value of 0.05 or less was considered significant. SPSS version 19 was used for the analysis.

RESULTS

Characteristics of the study subjects

The characteristics of the children analyzed in this study are listed in Table 1. Although children with NAA tended to be younger and shorter than those with NAC, there were no differences in age, sex distribution, height, weight, and body mass index between the 2 asthma groups. The asthma groups did not show any differences in age at the onset of symptoms or the severity of asthma. The prevalence of parental asthma history was significantly higher in the NAA group than in NAC group. However, there was no statistical difference between both asthma groups (Table 1).

Comparison of lung function, BHR, and eNO between the AA and NAA groups

We examined lung function and BHR in all the 3 study groups. While there were no significant differences in FEV1 % pred and FVC % pred values between the 3 groups, the FEV1/FVC and

FEF_{25%-75%} % pred levels were significantly reduced in both asthma groups compared to the control group (*P*<0.05). However, there were no differences in FEV1/FVC and FEF_{25%-75%} % pred values between both asthma groups.

Both asthma groups showed higher DRS methacholine levels than the NAC group (*P*<0.01), without significant differences between both asthma groups. The DRS AMP levels were higher in the AA group than the NAA or NAC group (*P*<0.01 for each). The DRS AMP levels in the NAA group did not differ from those in the NAC group.

The eNO levels were significantly higher in the AA group than in the NAA or NAC groups, respectively (*P*<0.01 for each). These differences were not observed between the NAA and NAC groups. The AA group showed the highest blood eosinophil counts among the 3 study groups (AA vs NAA, *P*<0.05; AA vs NAC, *P*<0.01). The NAA group showed a higher blood eosinophil level than the NAC group (*P*<0.05). Likewise, the serum total IgE levels were higher in the AA group than either the NAA or NAC group, respectively (*P*<0.01 for each). The serum total IgE levels in the NAA group did not significantly differ from those in the NAC group (Table 2).

Relationships between lung function, BHR, and eNO levels in the AA group

In the AA group, the FEV1 % pred measurement showed a significant negative correlation with DRS methacholine (*r*= -0.280, *P*<0.01), a marginally significant negative correlation with DRS AMP (*r*= -0.192, *P*=0.064). The levels of FEF_{25%-75%} % pred had a significant negative correlation with DRS methacholine (*r*= -0.301, *P*<0.01) and a significant negative correlation with DRS AMP (*r*= -0.206, *P*<0.05). Moreover, the eNO levels showed a significant negative correlation with FEV1/FVC (*r*= -0.246,

Table 1. Baseline characteristics of the subjects in this study

	Atopic asthma (n=100)	Non-atopic asthma (n=36)	Non-atopic control (n=40)
Age (year)	8.2 \pm 3.0	7.4 \pm 2.5*	9.1 \pm 2.9
Males/females	62/38	18/18	24/16
Height (cm)	128.2 \pm 17.7	124.3 \pm 16.6*	135.2 \pm 18.2
Weight (kg)	30.0 \pm 13.6	29.3 \pm 16.1	33.3 \pm 12.9
BMI (kg/m ²)	17.3 \pm 3.3	17.9 \pm 3.7	17.5 \pm 2.6
Age at onset of symptoms (year)	5.0 \pm 2.6	4.5 \pm 3.1	NA
Asthma severity, n (%)			
Mild intermittent	74 (74)	24 (66.7)	
Mild persistent	17 (17)	9 (25.0)	
Moderate persistent	8 (8)	3 (8.3)	
Severe persistent	1 (1)	0 (0)	
Allergic rhinitis	69 (69)	0 (0)	0 (0)
Parental asthma, MD diagnosis, n (%)	8 (8.3)	6 (18.2)*	1 (2.5)

Data are the mean \pm SD unless otherwise stated.

**P*<0.05 vs non-atopic controls.

BMI, body mass index; MD, medical doctor.

Table 2. Lung function, bronchial hyperresponsiveness, and exhaled nitric oxide measurements among the 3 study groups

	Atopic asthma (n=100)	Non-atopic asthma (n=36)	Non-atopic control (n=40)
FEV1 % pred	107.8 ± 19.4	108.7 ± 20.4	110.9 ± 17.8
FVC % pred	117.9 ± 18.8	120.1 ± 21.8	116.4 ± 17.7
FEV1/FVC % pred	87.4 ± 7.6*	87.0 ± 8.8*	91.4 ± 4.6
FEF _{25%-75%} % pred	81.8 ± 27.9*	81.5 ± 25.9*	96.7 ± 24.5
DRS methacholine	0.98 (0.69-1.38) [†]	0.91(0.69-1.20) [†]	0.62 (0.51-0.75)
DRS AMP	0.67 (0.51-0.88) [†]	0.57 (0.46-0.71)	0.51 (0.51-0.52)
Exhaled nitric oxide, ppb	23.8 (12.1-46.8) [†]	13.4 (6.8-26.3)	10.6 (6.1-18.4)
Blood eosinophils (/mm ³)	424.2 (210.4-855.2) [§]	272.9 (104.2-715.2)*	169.7 (83.9-343.2)
Total IgE (kU/L)	354.2 (124.0-1011.8) [†]	70.3 (16.8-293.6)	51.9 (17.6-153.3)

Data are the mean ± SD or geometric mean (range of 1 SD).

**P*<0.05 vs non-atopic controls; [†]*P*<0.01 vs non-atopic controls; [‡]*P*<0.01 vs non-atopic asthmatic children; [§]*P*<0.05 vs non-atopic asthmatic children.

FEV1, forced expiratory volume in one second; % pred, percentage predicted; FVC, forced vital capacity; FEF_{25%-75%}, forced expiratory flow at 25% to 75% of the FVC; DRS, dose-response slope; AMP, adenosine 5'-monophosphate.

Table 3. Correlations among lung function, bronchial hyperresponsiveness and exhaled nitric oxide, and total IgE in children with atopic asthma (AA) and non-atopic asthma (NAA)

Variables	FEF _{25%-75%} % pred		DRS methacholine		DRS AMP		eNO	
	AA	NAA	AA	NAA	AA	NAA	AA	NAA
DRS methacholine	-0.301*	-0.354 [†]	-	-	-	-	-	-
DRS AMP	-0.206 [‡]	-0.158	0.527 [§]	0.430 [‡]	-	-	-	-
eNO	-0.150	0.231	0.259 [‡]	0.291	0.357 [§]	0.217	-	-
Total IgE	-0.009	0.020	0.033	0.116	-0.008	0.087	0.291*	0.146

Data are correlation coefficient (Pearson's *r*) with controls for sex and height.

**P*<0.01; [†]*P*=0.051; [‡]*P*<0.05; [§]*P*<0.001.

AA, atopic asthma; NAA, non-atopic asthma; FEF_{25%-75%}, forced expiratory flow at 25% to 75% of the FVC; DRS, dose-response slope; AMP, adenosine 5'-monophosphate; eNO, exhaled nitric oxide.

P<0.05), but not with FEV1 % pred (*r*=-0.108) or FEF_{25%-75%} % pred (*r*=-0.150).

In our analyses of the association between the eNO levels and BHR indices, we found significant positive correlations with DRS methacholine (*r*=0.259, *P*<0.05) and DRS AMP (*r*=0.357, *P*<0.001).

In the association of the BHR indices and eNO with atopic markers, the DRS AMP values showed a significant correlation with the sum of wheals (*r*=0.378, *P*<0.001), but not with serum total IgE (*r*=-0.008). DRS methacholine did not have a significant correlation with atopic inflammatory markers (sum of wheals, *r*=0.164; serum total IgE, *r*=0.033). The levels of eNO showed a significant positive correlation with the serum total IgE (*r*=0.291, *P*<0.01) and a marginally significant correlation with the sum of wheals (*r*=0.186, *P*=0.073) (Table 3).

Relationships between lung function, BHR, and eNO in the NAA group

In the NAA group, the lung function parameters showed no significant correlation with BHR indices or the eNO levels with the exception of FEF_{25%-75%} % pred. The FEV1 % pred values had

no significant correlation with any BHR indices (DRS methacholine, *r*=-0.259; DRS AMP, *r*=-0.201). The levels of FEF_{25%-75%} % pred were found to have a marginally significant negative correlation with DRS methacholine (*r*=-0.354, *P*=0.051), but no significant correlation with DRS AMP (*r*=-0.158).

The levels of eNO did not correlate with any of the lung function parameters tested (FEV1 % pred, *r*=0.186; FEV1/FVC, *r*=0.122; FEF_{25%-75%} % pred, *r*=0.231) or any BHR indices (DRS methacholine, *r*=0.291; DRS AMP, *r*=0.217).

When we evaluated the association of the BHR index and eNO with the atopic markers, BHR to methacholine or AMP had no significant correlation with serum total IgE (DRS methacholine, *r*=0.116; DRS AMP, *r*=0.087). The eNO levels had no significant correlation with serum total IgE (*r*=0.146) (Table 3).

DISCUSSION

In our study, children with AA and NAA were found to share some common features including reduced small airway function, and increased BHR to methacholine challenge. However, the children with AA showed higher BHR to AMP and eNO lev-

els than the NAA children. When relationships among airway inflammation, BHR, and lung function were evaluated, the AA group showed significant relationships between lung function, BHR to AMP or methacholine, and the eNO levels. However, the NAA group showed only an association between small airway function and BHR to methacholine.

In this study, BHR to AMP was found to be higher in children with AA than with NAA. In contrast, BHR to methacholine was found not to differ between the 2 groups. Moreover, AMP responsiveness was also associated with the degree of atopy in children with AA. Recently, Suh *et al.*⁹ have reported that BHR to AMP is greater in children with AA than in those with NAA and associated with the degree of atopy, whereas BHR to methacholine does not differ between the 2 groups, which is consistent with our findings. These results collectively suggest that children with AA have airways that are more responsive to AMP than to methacholine and that this response increases with the degree of atopy in a dose-dependent manner. However, the pathogenesis of methacholine responsiveness may involve mechanisms other than atopy. In this study, children with AA also showed higher levels of eNO and blood eosinophils than those with NAA. Increased eNO levels and sputum eosinophils were previously shown to be detected in AA, not in NAA.^{7,11} In addition, previous studies have reported that eNO levels are related to eosinophil counts in sputum and lavage fluid.^{21,22} These results suggest that eosinophilic airway inflammation could be responsible for higher NO production in children with AA.

In our AA cases, BHR to methacholine was found to be associated with eNO levels, as well as baseline FEV1 and FEF_{25%-75%}. BHR to methacholine, a direct stimulus, has been considered to have 2 components: variable and fixed.²³ Whereas variable components can be the eNO level, a marker of airway inflammation, fixed components can be baseline FEV1 and FEF_{25%-75%} values, indices that reflect airway structural and functional changes in this study. Similarly, BHR to AMP, an indirect stimulus, was also found to be associated with baseline lung function as well as eNO levels in patients with AA. In addition, eNO levels were associated with the degree of atopic parameters, such as serum IgE levels in patients with AA. These findings collectively suggest that there are interactions between key features of atopic asthma, including atopy, inflammation, and BHR. Previous findings that the relationship between eNO and BHR was only found in atopic children^{24,25} were consistent with our results in this regard.

In contrast to the aforementioned findings in patients with AA, children in our NAA group showed only an association between small airway function and BHR to methacholine. No relationship in the NAA cases was found among airway inflammation and BHR to methacholine or AMP in contrast with the findings in the AA cases. We thus speculate that mechanisms other than atopic airway inflammation underlie the pathogen-

esis of BHR to methacholine in patients with NAA. Mochizuki *et al.*²⁶ have previously reported that the speed of bronchoconstriction to methacholine is higher in children with NAA than in those with AA, which suggests the mechanism of BHR may differ between those with AA and NAA. As another mechanism, we can speculate that certain harmful stimulations like lower respiratory tract infections in early childhood can alter the airway structure leading to airway remodeling processes that are considered to induce airway tone regulation failure and airway obstruction, regardless of eosinophilic airway inflammation, which was demonstrated by previous epidemiologic studies.^{3,27} Skevachi *et al.*²⁸ have demonstrated that rhinovirus can induce basic fibroblast growth factors and stimulate lung fibroblast proliferation, which may contribute to airway remodeling. Piedimonte has proposed that respiratory syncytial virus (RSV) infection in early life increases nerve growth factors and neurotrophin receptor expression that cause abnormal remodeling of neuronal networks in the respiratory tract resulting in BHR and airway obstruction.²⁹ Finally, neutrophilic airway inflammation in induced sputum has recently been reported to be a main feature in children with NAA,¹⁰ and this inflammation may not be represented by BHR to AMP or eNO. The findings in these previous studies can explain why our patients with NAA have a similar degree of BHR to methacholine, but a lower degree of BHR to AMP, compared to those with AA and show only an association of BHR to methacholine with small airway function, but not with the eNO levels in our study.

Different associations of lung function, bronchial hyperresponsiveness, and exhaled nitric oxide between subjects with AA and NAA may be caused by a selection bias. However, we did not select children with AA and NAA separately. Subjects with asthma were recruited irrespective of atopic status during study periods and then classified into those with AA and NAA based on SPT results. The proportion of atopy in those with asthma was 73.5% (36/100), which is similar to proportions of 72.7%-73.0% found in previous hospital-based studies in Korean children with asthma.^{30,31} However, it is too simple to classify asthma phenotypes into NAA and AA on the basis of SPT results as most previous studies^{6,10} and our study used. Childhood NAA may comprise a more heterogeneous group of disorders than AA, which may have structural changes in the airway after viral infection during early childhood, eosinophilic airway inflammation similar to AA, and non-eosinophilic (neutrophilic) airway inflammation as mentioned above. Further research is needed to classify asthma endotypes during childhood on the basis of the mechanism.

An AMP challenge was performed after a methacholine challenge on the same day in our asthmatic children. It is unlikely that the methacholine challenge influenced the results of the AMP challenge, because we performed the AMP challenge when the baseline FEV1 had recovered to within 5% of the methacholine challenge baseline without any respiratory

symptoms suggestive of airway obstruction. Hence, the baseline airway calibers after the methacholine challenge may not have exerted an effect on the PC20 of the AMP challenge. In these experiments, baseline FEV1 values in the 2 challenges were not significantly different (data not shown).

To our knowledge, our study is the first to report that relationships between lung function, BHR to direct or indirect stimulus, and airway inflammation differ between children with AA and NAA. Relationships were clearly evident between lung function, airway inflammation, and BHR in children with AA. However, these relationships were not found in children with NAA, with the exception of small airway function and BHR to methacholine. Our findings thus suggest that there is a different pathophysiology between AA and NAA in terms of relationships between lung function, airway inflammation, and BHR, which may have implications for the selection of therapeutic strategies, such as inhaled steroids, in these children. However, further studies are needed to clarify whether response to the same therapeutic strategy is different between AA and NAA before possible clinical implications can be drawn.

REFERENCES

- Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. *Allergy* 2012;67:835-46.
- Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;332:133-8.
- Stein RT, Holberg CJ, Morgan WJ, Wright AL, Lombardi E, Taussig L, et al. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. *Thorax* 1997;52:946-52.
- Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;368:763-70.
- Kelley CF, Mannino DM, Homa DM, Savage-Brown A, Holguin F. Asthma phenotypes, risk factors, and measures of severity in a national sample of US children. *Pediatrics* 2005;115:726-31.
- Castro-Rodriguez JA, Ramirez AM, Toche P, Pavon D, Perez MA, Girardi G, et al. Clinical, functional, and epidemiological differences between atopic and nonatopic asthmatic children from a tertiary care hospital in a developing country. *Ann Allergy Asthma Immunol* 2007;98:239-44.
- Gratziau C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur Respir J* 1999;14:897-901.
- Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. *Am J Respir Crit Care Med* 2008;178:476-82.
- Suh DI, Lee JK, Kim CK, Koh YY. Bronchial hyperresponsiveness to methacholine and adenosine 5'-monophosphate, and the presence and degree of atopy in young children with asthma. *Clin Exp Allergy* 2011;41:338-45.
- Drews AC, Pizzichini MM, Pizzichini E, Pereira MU, Pitrez PM, Jones MH, et al. Neutrophilic airway inflammation is a main feature of induced sputum in nonatopic asthmatic children. *Allergy* 2009;64:1597-601.
- Ryttilä P, Pelkonen AS, Metso T, Nikander K, Haahtela T, Turpeinen M. Induced sputum in children with newly diagnosed mild asthma: the effect of 6 months of treatment with budesonide or disodium cromoglycate. *Allergy* 2004;59:839-44.
- Cockcroft D, Davis B. Direct and indirect challenges in the clinical assessment of asthma. *Ann Allergy Asthma Immunol* 2009;103:363-9.
- Van Den Berge M, Meijer RJ, Kerstjens HA, de Reus DM, Koëter 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.
- 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.
- Munakata M. Exhaled nitric oxide (FeNO) as a non-invasive marker of airway inflammation. *Allergol Int* 2012;61:365-72.
- Prieto L, Esnal S, Lopez V, Barato D, Rojas R, Marín J. Maximal response plateau to adenosine 5'-monophosphate in asthma. Relationship with the response to methacholine, exhaled nitric oxide, and exhaled breath condensate pH. *Chest* 2009;135:1521-6.
- 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.
- Song DJ, Han YN, Lee JH, Kim HJ, Lim JY, Pee DH, et al. Lung function reference values in healthy Korean children. *Pediatr Allergy Respir Dis* 2002;12:105-13.
- Chinn S. Methodology of bronchial responsiveness. *Thorax* 1998;53:984-8.
- 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.
- Piacentini GL, Bodini A, Costella S, Vicentini L, Mazzi P, Sperandio S, et al. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. *Eur Respir J* 1999;13:1386-90.
- Fitzpatrick AM, Brown LA, Holguin F, Teague WG; National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. Levels of nitric oxide oxidation products are increased in the epithelial lining fluid of children with persistent asthma. *J Allergy Clin Immunol* 2009;124:990-6.e1-9.
- Cockcroft DW. Direct challenge tests: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010;138:18S-24S.
- Franklin PJ, Turner SW, Le Souëf PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. *Thorax* 2003;58:1048-52.
- Lúdvíksdóttir D, Janson C, Björnsson E, Stålenheim G, Boman G, Hedenström H, et al. Different airway responsiveness profiles in atopic asthma, nonatopic asthma, and Sjögren's syndrome. BHR Study Group. Bronchial hyperresponsiveness. *Allergy* 2000;55:259-65.
- Mochizuki H, Shigeta M, Tokuyama K, Morikawa A. Difference in airway reactivity in children with atopic vs nonatopic asthma. *Chest* 1999;116:619-24.
- Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig

- LM, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541-5.
28. Skevaki CL, Psarras S, Volonaki E, Pratsinis H, Spyridaki IS, Gaga M, et al. Rhinovirus-induced basic fibroblast growth factor release mediates airway remodeling features. *Clin Transl Allergy* 2012;2:14.
29. Piedimonte G. Contribution of neuroimmune mechanisms to airway inflammation and remodeling during and after respiratory syncytial virus infection. *Pediatr Infect Dis J* 2003;22:S66-74.
30. Son JW, Kim YK, Chung JW, Lee SR, Cho SH, Min KU, et al. Comparison of basophil histamine releasability between atopic and nonatopic asthmatics. *J Korean Med Sci* 1999;14:153-8.
31. Hong SJ, Lee SY, Kim HB, Kim JH, Kim BS, Choi SO, et al. IL-5 and thromboxane A2 receptor gene polymorphisms are associated with decreased pulmonary function in Korean children with atopic asthma. *J Allergy Clin Immunol* 2005;115:758-63.