

REGULAR ARTICLE

# Clinical characteristics of eosinophilic and noneosinophilic asthma in children

Yong Ju Lee<sup>1</sup>, Kyung Won Kim<sup>2</sup>, Bong Seok Choi<sup>3</sup>, Myung Hyun Sohn (mhsohn@yuhs.ac)<sup>2</sup>, Kyu-Earn Kim<sup>2</sup>

1.Department of Pediatrics, Kangnam Sacred Heart Hospital, Hallym University, Seoul, Korea

2.Department of Pediatrics and Institute of Allergy, Severance Medical Research Institute, Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul, Korea

3.Department of Pediatrics, Good Gangan Hospital, Busan, Korea

## Keywords

Airway inflammation, Asthma in children, Eosinophils, Neutrophils

## Correspondence

MH Sohn, Department of Pediatrics, Yonsei University College of Medicine, 250 Seongsan-ro, Seodaemun-gu, Seoul 120-752, Korea.

Tel: +82-2-2228-2062 |

Fax: +82-2-393-9118 |

Email: mhsohn@yuhs.ac

## Received

3 June 2012; revised 24 September 2012; accepted 28 September 2012.

DOI:10.1111/apa.12046

The first two authors contributed equally to this work.

## ABSTRACT

**Aim:** Asthma is a heterogeneous and complex chronic inflammatory disease of the airways. Asthma can be classified as eosinophilic asthma (EA) or noneosinophilic asthma (NEA). We investigated whether children with EA manifest different clinical characteristics than those with NEA.

**Methods:** We enrolled 288 steroid-naïve asthmatic children and classified them, based on the cell counts in induced sputum, into EA (158 children) and NEA (89 children) groups.

**Results:** No significant differences were observed between the groups with regard to age, sex, family history of atopy, secondary smoking or asthma exacerbations. Moderate-to-severe asthma was more frequent in the EA group than in the NEA group. Blood eosinophil counts and serum eosinophil cationic protein were higher in EA patients than in NEA patients. The forced expiratory volume in 1 sec was lower in children with EA than in those with NEA (% of predicted value,  $88.6 \pm 18.5$  vs.  $93.6 \pm 15.6$ ,  $p < 0.05$ ). The sputum eosinophil (in EA) and neutrophil (in NEA) counts increased with increasing asthma severity.

**Conclusion:** Airway inflammation, especially eosinophilic inflammation, was associated with asthma severity and reduced pulmonary function in children.

## INTRODUCTION

Asthma is a heterogeneous disease characterized by various degrees of airway obstruction, airway hyper-responsiveness (AHR) and chronic airway inflammation. There are several biologic markers for the diagnosis and assessment of asthma, including blood eosinophil levels, serum eosinophil cationic protein (ECP), exhaled nitric oxide and induced sputum. Among these biomarkers, induced sputum is useful parameter for assessing airway inflammation because it reflects both upper and lower airway inflammation (1). Asthma can be classified as eosinophilic asthma (EA) or noneosinophilic asthma (NEA) based on the induced sputum (2).

In adults, EA, as opposed to NEA, has been associated with more severe asthma (3), more severe airway obstruction, increased airway reactivity to methacholine (4) and a better response to treatment with inhaled corticosteroids (ICS) (5). NEA has been reported to be associated with refractory asthma (5), pollutants, smoking, occupational allergens and obesity (2). However, a recent study has shown that the patterns of the inflammatory phenotypes differ between adults and children (6), and there are only a few studies concerning the relationship between clinical characteristics and airway inflammation. In children,

cough frequency (7) and nonatopic asthma (8) were associated with sputum neutrophilia, while frequent asthma exacerbations were associated with sputum eosinophilia (9).

In this study, we investigated whether children with EA and those with NEA (as defined by the inflammatory patterns of induced sputum) manifested with different clinical characteristics. Additionally, we studied the extent to which airway inflammation in each phenotype was associated with asthma severity, pulmonary function and AHR.

## Key notes

- Moderate-to-severe symptoms are more frequent in eosinophilic asthma (EA) than in noneosinophilic asthma (NEA) in children.
- Eosinophilic inflammation is associated with severity of EA.
- Neutrophilic inflammation is associated with severity of NEA.

## PATIENTS AND METHODS

### Patients

This study enrolled 288 children who visited the Asthma and Allergy Clinic at the Severance Children's Hospital from July 2006 to July 2009 to confirm asthma because of recurrent symptoms of asthma. Their ages ranged from 6 to 15 years. The inclusion criteria were recurrent symptoms of wheezing, dyspnoea or chest tightness during the previous year, a provocative concentration of methacholine causing a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub>) of <16 mg/mL or a change in forced expiratory volume in 1 s (FEV<sub>1</sub>) of  $\geq 12\%$  between pre- and postbronchodilator (BD) inhalation. The symptoms were reported by the parents. The exclusion criteria were the use of inhaled or systemic corticosteroids or leukotriene receptor antagonists, infection of the respiratory tract within 1 month of enrolment and refusal to undergo sputum test or inability to produce adequate sputum. This study was approved by the Institutional Review Board of the Severance Hospital. Written consent for participation was obtained from the parents with verbal assent from the children.

Forty-one children failed to produce adequate sputum and were excluded from the analysis. Their average age was  $7.6 (\text{mean}) \pm 1.8$  (standard deviation) years, compared with  $8.7 \pm 2.8$  years in attending children ( $p < 0.05$ ). Their clinical characteristics, blood eosinophil levels, serum ECP, pulmonary function results and PC<sub>20</sub> values were not significantly different from those of NEA patients.

### Methods

Sputum eosinophilia was defined as eosinophils comprising >2.5% of the cells counted in induced sputum (10). Patients with sputum eosinophilia were classified as having EA and patients without sputum eosinophilia as having NEA.

Asthma severity was classified as intermittent, mild persistent or moderate-to-severe persistent based on the extent of symptoms and the pulmonary function results. Patients in the intermittent group had daytime symptoms less than or once a week, nocturnal symptoms less than or twice a month and pre-BD FEV<sub>1</sub> (% of predicted value)  $\geq 80\%$ . The mild persistent group had day symptoms more than once a week but less than once a day, nocturnal symptoms more than twice a month and pre-BD FEV<sub>1</sub> (%)  $\geq 80\%$ . The moderate-to-severe persistent asthma patients had symptoms daily, nocturnal symptoms more than once a week and pre-BD FEV<sub>1</sub> (%) < 80%. Atopy was defined by a positive skin prick test upon exposure to one or more extracts of 12 common aeroallergens or a serum total immunoglobulin E (IgE) concentration of  $\geq 150$  IU/mL. Nonatopy was defined by a negative skin prick test with a serum total IgE concentration of <150 IU/mL (11). The allergens tested included *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat and dog epithelia, sagebrush, ragweed, oak, birch, *Aspergillus* spp., *Alternaria* spp. and American and German cockroach antigens (Torii & Co., Tokyo, Japan). Saline was used as a negative control, and 0.5% histamine HCl was used as a positive control. The wheal diameter was measured after 15 min, and a positive reaction was defined

as a wheal diameter  $\geq 3$  mm. A parental history of atopy was present when at least one parent had atopic dermatitis, asthma or allergic rhinitis.

The pulmonary function and methacholine challenge tests were performed on different days and when the patients were stable and without acute exacerbation. A pulmonary function test (VIASYS Healthcare Inc., Conshohocken, PA, USA) was performed according to ATS guidelines before and after BD inhalation (12). A methacholine challenge test was performed according to a standardized method (13). Each subject inhaled increasing concentrations of methacholine (0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10, and 25 mg/mL) nebulized by a Mefar MB3 dosimeter (Brescia, Italy) until FEV<sub>1</sub> was reduced by 20% from a postnebulized saline value. Bronchial response to methacholine was expressed as a provocative concentration of methacholine causing a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub> in mg/mL) and was calculated by linear interpolation of the log dose-response curve (14). Patients with an estimated pre-BD FEV<sub>1</sub> (%) of <70% did not undergo the methacholine challenge test.

Sputum induction and processing were performed as previously described (14). The induced sputum was considered adequate if the patient inhaled 3% saline for longer than 4 min, the induced sputum volume was  $\geq 1$  mL, the sample contained >400 inflammatory cells and the proportion of squamous epithelial cells was  $\leq 80\%$  (15).

The NE-8000 system (Sysmex, Kobe, Japan) was used to count eosinophils automatically in peripheral blood, while serum total IgE, specific IgE and ECP were measured with the CAP system (Pharmacia-Upjohn, Uppsala, Sweden).

### Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (version 15.0; SPSS, Inc., Chicago, IL, USA). Numerical variables are expressed as means and standard deviations. Numeric data were compared using the two-sample *t*-test. Frequencies were compared using the chi-square test. Analysis of variance (ANOVA) was used to compare sputum inflammatory cells or pulmonary function among the asthma severity groups. A *p*-value of <0.05 was considered statistically significant.

## RESULTS

There were 158 children who had EA and 89 who had NEA. The frequency of moderate-to-severe persistent asthma in the EA group was greater than that in the NEA group (Table 1). Blood eosinophils and serum ECP were higher in the EA than in the NEA group (Table 2). Both pre-BD and post-BD FEV<sub>1</sub> were lower in the EA than in the NEA (Table 2). However, pre-BD FEV<sub>1</sub>/FVC values and changes in FEV<sub>1</sub> did not differ between the groups. When the lung function findings were compared separately in the subgroups constructed according to the severity of asthma, the differences between the EA and NEA groups were seen in children with mild asthma but not in those with moderate-to-severe asthma (data not shown).

**Table 1** Patient characteristics

| Characteristic                                                    | EA group<br>(n = 158) | NEA group<br>(n = 89) |
|-------------------------------------------------------------------|-----------------------|-----------------------|
| Age (year)                                                        | 8.6 ± 2.5             | 8.9 ± 3.4             |
| Sex [M/F (%)]                                                     | 94 (59)/64 (41)       | 60 (67)/29 (23)       |
| Atopy, n (%)                                                      | 137 (87)              | 69 (77)               |
| Parental history<br>of atopy, n (%)                               | 84 (53)               | 45 (51)               |
| Secondary smoking, n (%)                                          | 37 (23)               | 25 (28)               |
| Household pets, n (%)                                             | 9 (6)                 | 4 (5)                 |
| Number of asthma<br>exacerbations in the<br>last 12 months, n (%) |                       |                       |
| 0                                                                 | 101 (64)              | 62 (70)               |
| 1                                                                 | 41 (26)               | 20 (23)               |
| ≥ 2                                                               | 16 (10)               | 7 (7)                 |
| Asthma severity (%)*                                              |                       |                       |
| Intermittent                                                      | 26 (16)               | 27 (30)               |
| Mild persistent                                                   | 83 (52)               | 44 (50)               |
| Moderate-to-severe<br>persistent                                  | 49 (31)               | 18 (20)               |

EA, eosinophilic asthma; NEA, noneosinophilic asthma.

Values are presented as the mean ± standard deviation or absolute numbers. Numeric data were analysed using the two-sample *t*-test, and the frequency was analysed using chi-square test. Parental history of atopy was present if at least one of the parents had atopic dermatitis, asthma or allergic rhinitis.

\**p* < 0.05 vs. NEA.

**Table 2** Comparison between laboratory data and pulmonary function parameters

| Parameter                                  | EA group (n = 158) | NEA group (n = 89) |
|--------------------------------------------|--------------------|--------------------|
| Blood eosinophils (per $\mu$ L)            | 542 ± 343*         | 371 ± 338          |
| Total IgE (IU/mL)                          | 600 ± 681          | 529 ± 609          |
| Serum ECP ( $\mu$ g/L)                     | 46.6 ± 43.6*       | 30.4 ± 36.7        |
| Number of positive skin tests              | 2.3 ± 1.7          | 2.1 ± 2.2          |
| Abnormal FEV <sub>1</sub> , n (%)          | 41 (26)            | 15 (17)            |
| Pre-BD FEV <sub>1</sub> (% predicted)      | 88.6 ± 18.5**      | 93.6 ± 15.6        |
| Pre-BD FEV <sub>1</sub> /FVC (%)           | 86.7 ± 11.2        | 88.3 ± 10.0        |
| Pre-BD FEF <sub>25–75%</sub> (% predicted) | 77.9 ± 30.6**      | 87.1 ± 31.1        |
| Post-BD FEV <sub>1</sub> (% predicted)     | 97.1 ± 18.3**      | 102.0 ± 15.8       |
| Change in FEV <sub>1</sub> (%)             | 10.7 ± 10.9        | 8.9 ± 20.2         |
| PC <sub>20</sub> (mg/mL)                   | 8.5 ± 12.9         | 10.8 ± 14.3        |

EA, eosinophilic asthma; NEA, noneosinophilic asthma; IgE, immunoglobulin E; ECP, eosinophil cationic protein; FEV<sub>1</sub>, forced expiratory volume in 1 s; Pre-BD, pre-bronchodilator; FVC, forced vital capacity; FEF<sub>25–75%</sub>, forced expiratory flow at the mid-portion of forced vital capacity; Post-BD, postbronchodilator; PC<sub>20</sub>, provocative concentration of methacholine causing a 20% decrease in FEV<sub>1</sub>.

Values are presented as the mean ± standard deviation or absolute numbers. Numeric data were analysed using the two-sample *t*-test, and the frequency was analysed using chi-square test. Abnormal FEV<sub>1</sub> was defined as Pre-BD FEV<sub>1</sub> (% predicted) below 80%.

\**p* < 0.01,

\*\**p* < 0.05, vs. NEA.

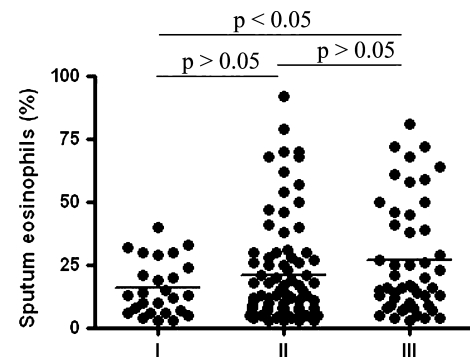


**Figure 1** Sputum eosinophils according to asthma severity in eosinophilic asthma (EA) patients. The means of the sputum eosinophil values in the intermittent, mild persistent and moderate-to-severe persistent asthma groups were  $17.4 \pm 12.7\%$ ,  $21.0 \pm 20.5\%$  and  $26.6 \pm 22.0\%$ , respectively. The moderate-to-severe persistent asthma group had a higher percentage of sputum eosinophils than the intermittent asthma group. I: intermittent asthma group; II: mild persistent asthma group; III: moderate-to-severe persistent asthma group. The horizontal lines represent mean values.

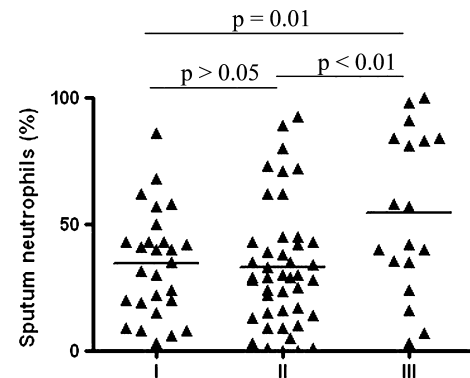


**Figure 2** Sputum neutrophils according to asthma severity in noneosinophilic asthma (NEA) patients. The means of the sputum neutrophil values in the intermittent, mild persistent and moderate-to-severe persistent asthma groups were  $34.2 \pm 21.0\%$ ,  $32.8 \pm 24.3\%$  and  $54.3 \pm 31.8\%$ , respectively. The moderate-to-severe persistent asthma group had a higher percentage of sputum eosinophils than the intermittent asthma group or the mild persistent asthma group. I: intermittent asthma group; II: mild persistent asthma group; III: moderate-to-severe persistent asthma group. The horizontal lines represent mean values.

The mean percentages of macrophages, neutrophils, eosinophils and lymphocytes in the induced sputum samples were  $46.7 \pm 25.4\%$  (mean ± standard deviation),  $30.6 \pm 22.6\%$ ,  $22.1 \pm 20\%$  and  $0.2 \pm 1\%$ , respectively, in the EA group, and  $61.8 \pm 6.4\%$  (*p* < 0.001),  $37.6 \pm 26.2\%$  (*p* < 0.05),  $0.4 \pm 0.6\%$  and  $0\%$  (*p* < 0.001), respectively, in the NEA group.

Sputum eosinophils increased with asthma severity in the EA patients (Fig. 1). However, there was no association between asthma severity and sputum neutrophils in the EA group (data not shown). Similar to sputum eosinophils in the EA patients, in the NEA patients, the percentage of sputum neutrophils in the moderate-to-severe persistent asthma group was higher than in the intermittent asthma and the mild persistent asthma groups (Fig. 2).

## DISCUSSION

In this study, we found that moderate-to-severe persistent asthma was more frequent in EA than in NEA and that children with EA had worse pulmonary function results than those with NEA. There were significant differences in levels of eosinophilic biomarkers between EA and NEA patients.

In the intermittent and mild persistent asthma groups, the pre-BD FEV<sub>1</sub>, pre-BD FEF<sub>25–75%</sub>, and post-BD FEV<sub>1</sub> were lower in the EA patients than in the NEA patients. However, we did not observe the same results in the moderate-to-severe persistent groups. This could indicate that airway inflammation decreases lung function in patients with severe asthma regardless of its pattern. It is also possible that factors other than asthma severity and airway inflammation, such as age of asthma onset, genetics, infection and lung structure, affect lung function in patients with severe asthma. The changes in FEV<sub>1</sub> and PC<sub>20</sub> did not differ between the groups. These findings probably indicate that eosinophilic inflammation affects pulmonary function rather than AHR or airway reversibility. On the other hand, pre-BD FEV<sub>1</sub>/FVC did not differ significantly between the groups. Most of the patients in the study had intermittent or mild persistent asthma and normal pulmonary function, which may be why the pre-BD FEV<sub>1</sub>/FVC results did not associate significantly with asthma severity in asthmatic children with normal pre-BD FEV<sub>1</sub> (16).

Eosinophilic airway inflammation is known to be associated with decreased pulmonary function, frequent exacerbations and poor prognosis (9,17). We used induced sputum to evaluate airway inflammation. According to previous studies, induced sputum has been a good tool for the evaluation of airway inflammation because it has been found to be highly correlated with bronchial wash results, but is less invasive (18,19). However, it is limited in that it is an indirect method. The exhaled nitric oxide (eNO) concentration has also been reported to be closely correlated with sputum eosinophil counts in adults with asthma (20) and can be useful to identify EA in a less invasive manner than induced sputum. This parameter was not investigated in the current study of paediatric asthma patients, and further studies are needed to evaluate its utility of eNO compared with induced sputum for the evaluation of airway inflammation.

In the present study, total blood eosinophil counts and serum ECP levels were higher in the EA patients than in the NEA patients. Blood eosinophil counts and serum ECP were weakly associated with sputum eosinophil counts in the whole study population, but not in the EA groups (data not shown). Blood eosinophil and serum ECP levels may also be increased in other allergic conditions, such as atopic dermatitis, and allergic rhinoconjunctivitis. Therefore, blood eosinophilic markers may have limited use as predictors of eosinophilic airway inflammation.

The ratios of patients with atopy, number of positive skin tests and total IgE levels were high and similar in both

groups. These findings were different from those previously reported, and NEA has been reported to be associated with nonatopic factors including pollutants, smoking, occupational allergens and obesity (2). In children, nonatopic asthma has been associated with sputum neutrophilia (8). This difference might have been because some patients were already sensitized to allergens but did not develop eosinophilic airway inflammation. The frequency of asthma exacerbation in the last year was low in both of our study groups because asthma exacerbation was defined as a condition that a patient needed systemic steroids to avoid hospitalization or intubation.

This study had a few limitations. First, we did not evaluate the reproducibility of the induced sputum. Although the induced sputum test is a simple method of evaluating airway inflammation, it is uncomfortable for children to perform it repeatedly. Moreover, many of our patients had mild persistent asthma but had not undergone treatment with ICS before this study. ICS were administered immediately after the tests, which were therefore not performed repeatedly. Second, we used the relative percentage of inflammatory cells in sputum to represent airway inflammation, without indicating the absolute cell counts. However, it has been suggested that absolute cell counts are relatively poorly reproducible and able to yield only limited information (21,22).

In conclusion, we found that pulmonary function in children with EA was more reduced than in those with NEA and that asthma severity was affected by the degree of eosinophilic inflammation in EA or neutrophilic inflammation in NEA.

## ACKNOWLEDGEMENTS

This research was supported by grant A090399 from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea.

## References

1. Lex C, Ferreira F, Zacharasiewicz A, Nicholson AG, Haslam PL, Wilson NM, et al. Airway eosinophilia in children with severe asthma: predictive values of noninvasive tests. *Am J Respir Crit Care Med* 2006; 174: 1286–91.
2. Haldar P, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. *J Allergy Clin Immunol* 2007; 119: 1043–52; quiz 53–4.
3. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 2000; 161: 9–16.
4. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol* 2001; 108: 753–8.
5. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; 160: 1001–8.

6. Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, et al. Different inflammatory phenotypes in adults and children with acute asthma. *Eur Respir J* 2011; 38: 567–74.
7. Li AM, Tsang TW, Chan DF, Lam HS, So HK, Sung RY, et al. Cough frequency in children with mild asthma correlates with sputum neutrophil count. *Thorax* 2006; 61: 747–50.
8. 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.
9. Lovett CJ, Whitehead BF, Gibson PG. Eosinophilic airway inflammation and the prognosis of childhood asthma. *Clin Exp Allergy* 2007; 37: 1594–601.
10. Gibson PG, Henry RL, Thomas P. Noninvasive assessment of airway inflammation in children: induced sputum, exhaled nitric oxide, and breath condensate. *Eur Respir J* 2000; 16: 1008–15.
11. Reefer AJ, Satinover SM, Wilson BB, Woodfolk JA. The relevance of microbial allergens to the IgE antibody repertoire in atopic and nonatopic eczema. *J Allergy Clin Immunol* 2007; 120: 156–63.
12. Crapo RO, Hankinson JL, Irvin CG, Macintyre NR, Voter KR, Wise RA, et al. Standardization of spirometry, 1994 update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152: 1107–36.
13. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; 161: 309–29.
14. Kim KW, Jee HM, Park YH, Choi BS, Sohn MH, Kim KE. Relationship between amphiregulin and airway inflammation in children with asthma and eosinophilic bronchitis. *Chest* 2009; 136: 805–10.
15. Covar RA, Spahn JD, Martin RJ, Silkoff PE, Sundstrom DA, Murphy J, et al. Safety and application of induced sputum analysis in childhood asthma. *J Allergy Clin Immunol* 2004; 114: 575–82.
16. Simon MR, Chinchilli VM, Phillips BR, Sorkness CA, Lemanske RF Jr, Szefer SJ, et al. Forced expiratory flow between 25% and 75% of vital capacity and FEV1/forced vital capacity ratio in relation to clinical and physiological parameters in asthmatic children with normal FEV1 values. *J Allergy Clin Immunol* 2010; 126: 527–34.e1-8.
17. Gibson PG, Simpson JL, Hankin R, Powell H, Henry RL. Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma. *Thorax* 2003; 58: 116–21.
18. Keatings VM, Evans DJ, O'Connor BJ, Barnes PJ. Cellular profiles in asthmatic airways: a comparison of induced sputum, bronchial washings, and bronchoalveolar lavage fluid. *Thorax* 1997; 52: 372–4.
19. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997; 52: 498–501.
20. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005; 35: 1175–9.
21. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; 11: 54–61.
22. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; 47: 25–9.