Word count (abstract): 201 Word count (text): 2491

**No evidence of airway inflammation in a large proportion of stable childhood asthma**

(Short title: Non-eosinophilic asthma in children)

Collin R Brooks,1 Christine J van Dalen,1 Angela Zacharasiewicz,2 Elizabeth Harding,1 Soo Cheng,1 Jodie Simpson,3,4 Jacquie L Harper,5 Graham Le Gros,5 Peter Gibson,3,4 Neil Pearce,1,6 Jeroen Douwes1

1 Centre for Public Health Research, Massey University, Wellington, New Zealand

2 Department of Pediatrics and Adolescent Medicine, Wilhelminenspital, Vienna, Austria

3 Respiratory and Sleep Medicine, School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

4 Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle NSW, Australia

5 Malaghan Institute of Medical Research, Wellington, New Zealand

6 Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom

*Correspondence:*

Jeroen Douwes, Centre for Public Health Research, Massey University,

Private Box 756, Wellington, New Zealand

E-mail: J.Douwes@massey.ac.nz Phone: +64 4 38006017, Fax: +64 4 8027120

**ABSTRACT**

**Background:**Neutrophilic inflammation has been implicated in non-eosinophilic asthma (NEA) in adults, but little is known about NEA in children.

**Aim:** To assess clinical and inflammatory characteristics of NEA in childhood asthma.

**Methods:** Airway inflammation, sputum endotoxin, airway hyperreactivity, atopy and lung function were assessed in 77 asthmatic and 68 non-asthmatic adolescents (12–17 years). Asthma was based on the presence or absence of wheeze and/or asthma history.

**Results:** The proportion of NEA (sputum eosinophils <2.5%) was 54%. In this group, atopy, sputum neutrophil, eosinophil, eosinophil cationic protein (ECP), endotoxin, neutrophil elastase and IL-8 levels were not different from non-asthmatics. In contrast, eosinophilic asthma (EA) was associated with atopy and sputum ECP and IL-8. The majority of NEA had no evidence of inflammation; only 14% had neutrophilia (≥61% neutrophils), compared with 11% of EA, and 15% of non-asthmatics. Small differences in FEV1 (NS) were found between EA and NEA, but symptom prevalence and severity was not different (83% of EA and 79% of NEA were classified moderate-to-severe).

**Conclusions:** NEA is common in childhood asthma and has similar clinical characteristics as EA. Neutrophils do not appear to play a role in NEA in children and underlying mechanisms may not involve airway inflammation.

**Summary at a Glance**

Our study suggests that many children with stable asthma do not appear to correspond to the conventional notion that asthma is ‘an inflammatory disorder of the airways’. In the absence of overt inflammation, the mechanisms responsible for asthma symptoms in a large proportion of childhood asthma remain unknown.

**Keywords:** airway inflammation;asthma; children; eosinophil; induced sputum

**Disclosure of potential conflict of interest**

P.G.Gibson has received honoraria from AstroZeneca, GlaxoSmithKline and Novartis. The remaining authors have no conflict of interest.

**INTRODUCTION**

Asthma is often considered to be an allergic disease involving TH2-mediated eosinophilic airways inflammation.1 However, there is increasing evidence that non-allergic mechanisms may also be important. We have previously proposed that “only” 50% of asthma cases are attributable to eosinophilic airway inflammation, and that in many cases non-eosinophilic asthma (NEA) may be due to neutrophilic inflammation.2 Other studies also suggest a possible role for neutrophils in adult NEA as reflected by increased airway neutrophil levels and associated chemokines including interleukin (IL)-8, neutrophil elastase (NE), matrix metalloprotease (MMP)-9,3-5 increased gene expression of innate immune receptors and levels of sputum bacterial endotoxin.6 However, neutrophilic inflammation in NEA is not always present,7 indicating that neutrophils may be important in only a proportion of NEA. In particular, we have previously observed no overt evidence of *either* eosinophilic or neutrophilic airway inflammation in over 30% of adult asthmatics.3

Studies in adult asthmatics have suggested that eosinophilic asthma (EA) is associated with increased clinical severity8 and exacerbation frequency9 compared with NEA although this is not always observed.3,7 There may also be pathological differences i.e. NEA appears to be less atopic, have normal sub-epithelial layer thickness and have poor short-term response to inhaled corticosteroids (ICS).5 Thus EA and NEA may represent distinct pathophysiological phenotypes with potentially different causative triggers and treatment strategies.

There are similar conflicting data in the limited number of studies which have studied NEA in children. In particular, EA is associated with significantly reduced lung function,10 increased airway hyperreactivity (AHR)11 and uncontrolled asthma12 in some studies, but there are also reports finding no significant clinical differences between EA and NEA.13,14 Equivocal findings have also been reported regarding the importance of neutrophils in childhood NEA.14-17

The aims of this study were to assess: 1) the importance of the NEA phenotype in children; 2) the contribution of airway neutrophils, associated inflammatory mediators, and bacterial endotoxin (previously associated with neutrophilic inflammation in adults6) in childhood NEA; and 3) whether childhood EA and NEA differ with regard to clinical parameters such as atopy, lung function, AHR, medication use, and severity.

**MATERIALS & METHODS**

**Study population**

We recruited students aged 12-17 years who previously participated in the ISAAC Phase III survey.18 Those who responded positively to the following questions were invited: “*Have you had wheezing or whistling in the chest in the past 12 months?*”, and “*Have you ever had asthma?*” (n=244); a random sample of those without respiratory symptoms were also invited (n=595). Those who agreed to participate (136 with and 134 without symptoms) completed a second questionnaire based on the ISAAC Phase III survey. Approval was obtained from the Wellington Ethics Committee (00/03/010). Written consent was obtained from children and their parents.

**Asthma definition**

Asthma was defined based on a positive response to the questions above, and/or asthma medication use in the past 12 months. Non-asthmatics had no wheezing/whistling in the chest or asthma medication in the last 12 months, no asthma history, and no nocturnal cough in the last 12 months apart from that associated with respiratory infection. Sixteen subjects were excluded because they did not meet eligibility criteria (either no current symptoms despite a history of asthma or an ambiguous questionnaire response). Eight subjects were unable to be re-contacted and excluded.

**Classification of asthma severity**

Asthma cases were defined by wheezing attack frequency per month (mild intermittent, 0-3; persistent-moderate, 4-12; severe, >12), sleep disturbance due to wheeze (mild, < 1 night/week; moderate, ≥ 1 night/week), speech limitation by wheeze in the past 12 months (severe), daily symptoms in last two weeks (moderate), daily use of β2 agonist (moderate), and FEV1 (mild, ≥ 80% predicted; moderate, ≥ 60% and < 80%; severe, < 60%) following 2008 GINA guidelines (for observational studies rather than clinical management).19 Subjects were assigned an asthma severity category according to the highest severity class for any parameter.

**Study procedures**

All study participants underwent a standard clinical assessment. Participants who had a respiratory infection within four weeks were asked to return at a later date. Atopy was defined as a positive skin prick test to at least one of of eight allergens, and lung function was measured following standard procedures (see supplement).20 Eleven subjects were unable to perform adequate spirometry, and 11 were excluded because of β2-agonist use within 6 hours prior to assessment.

Combined bronchial provocation and sputum induction using 4.5% saline were conducted as previously described (see supplement).11 Subjects with sputum eosinophils ≥2.5% were classified as EA with the remainder classified as NEA.21 Further phenotyping on the basis of sputum neutrophils ≥ 61% was also conducted.3 Seventy eight (35%) subjects failed to provide an adequate sputum sample (unable to conduct an inflammatory cell count), therefore complete data was available for 77 asthmatics and 68 non-asthmatics.

Eosinophil cationic protein and IL-8 in sputum supernatant were measured by ELISA (R&D Systems, Minneapolis, MN). Active NE was measured using n-methoxysuccinyl-l-alanyl-l-alanyl-prolyl-l-valyl-p-nitroanilide (Sigma, St Louis, MO, USA). Endotoxin was measured using the *Limulus* Amebocyte Lysate assay (Kinetic QCL; BioWhittaker, Walkersville, MD). These assays have previously been validated for assessment of induced sputum.6

**Statistical analyses**

Lung function measures were summarised as mean values with 95% confidence intervals (95% CI). Sputum cell counts, inflammatory markers and endotoxin were expressed as median values with 25th/75th-percentiles. Chi-square and t-tests were performed to test differences in prevalence and mean levels (with normally distributed data), respectively. The Wilcoxon rank-sum test was used for non-parametric data. Prevalence odds ratios (ORs) were calculated using logistic regression for analyses involving dichotomous outcomes; linear regression analyses were used for continuous outcomes. The population-attributable fraction (PAF) was calculated to assess the proportion of asthma attributable to eosinophilia. Data were analysed using SAS (SAS Institute, Cary, NC) and STATA 11 (Statacorp, College Station, TX).

**RESULTS**

Asthmatics and non-asthmatics did not differ with regards to age, sex and ethnicity, but asthmatics had, as expected, a small but significantly reduced FEV1 and FEV1/FVC, and significantly increased prevalence of atopy and AHR (Table 1). Approximately 40% of asthmatics were classified as severe, 40% as moderate and 20% as mild.

Fifty-four percent (n=42) of asthmatics had sputum eosinophil levels <2.5% and were designated NEA, with the remaining 46% designated EA. Eosinophil and ECP levels were significantly higher in EA than NEA or non-asthmatics (Figure 1A; Table 2), and those with EA were more likely to be atopic (Table 3). Eosinophil levels were similar in NEA and non-asthmatics (Figure 1B). Neutrophil levels were not significantly different between asthmatics and non-asthmatics (Figure 2A) or between EA and NEA (Figure 2B).

Thirty one children (40%) had EA in the absence of raised neutrophils and 10 (13%) had sputum neutrophil levels ≥61%. Of these, 6 (8%) were classified as neutrophilic asthma (NA) and 4 (5%) mixed granulocytic asthma (MGA). The remainder (n=36; 47%) had no evidence of granulocytic inflammation, and were classified as PGA (paucigranulocytic asthma). Table S1 shows the inflammatory and clinical characteristics of asthmatics stratified accordingly. Due to the small numbers of NA and MGA, further analyses were conducted using the NEA and EA classifications only.

Lung function in NEA was marginally (non-significantly) higher than in EA (Table 3). Similarly, AHR prevalence, asthma medication use and most symptoms were lower in NEA, but differences were small and not statistically significant. Although EA were slightly more likely to use ICS (Table 3), there were no significant differences in sputum eosinophil percentage when asthmatics were stratified by recent ICS use (data not shown). The prevalence of moderate-to-severe asthma was similar between both groups (83% and 79% in EA and NEA respectively), although 49% of EA were classified as severe compared with 33% of NEA (Table 3). Similarly, the EA group was more likely to have persistent asthma, although this was not significant (Table 3). Severity was not associated with eosinophils or neutrophils in all asthmatics or EA or NEA respectively (data not shown). Despite this, sputum eosinophils were inversely associated with lung function, reaching statistical significance for total eosinophils and FEV1 %-predicted (p<0.05; Table 4). Non-significant associations (p<0.10) were also found for %-eosinophils and FEV1-%-predicted as well as total eosinophils and FEV1/FVC-%- predicted (Table 4). Conversely, neutrophils were positively associated with FEV1-%-predicted and FVC-%-predicted.

Mediators associated with granulocytic inflammation (ECP, NE and IL-8) were inversely associated with FEV1/FVC-%-predicted, but not with other lung function variables (Table 4). Although undetectable in 62% (n=90) of samples, NE was correlated with neutrophils, but not with eosinophils (Table S2). No differences in NE levels were found between EA, NEA and non-asthmatics, although IL-8 was elevated in EA, but not in NEA (Table 2). Despite this, IL-8 was correlated with both eosinophils and neutrophils. ECP was strongly correlated with eosinophils (Table S2), and both were associated with atopy and AHR (Table 4). No differences were found in sputum endotoxin levels for any of the studied sub-groups (Table 2), and whilst endotoxin was weakly correlated with NE (r=0.17; p<0.05) (Table S2), it was not associated with lung function, atopy or AHR (Table 4). Associations were similar when analyses were restricted to only asthmatics, but results were generally no longer statistically significant. Adjusting analyses for asthma status, ICS use, or using a lower 54% neutrophil percentage cut-off to define airway neutrophilia12 did not significantly alter the results.

DISCUSSION

In this study NEA was slightly more common than EA. Those with NEA had marginally higher lung function, fewer symptoms and lower AHR, but differences were not statistically significant. The proportion of moderate-to-severe asthma in both groups was also similar, although severe asthma was slightly more common in EA. Increased neutrophil levels were not observed in NEA and no significant differences were found between NEA and non-asthmatics with regard to the inflammatory markers studied. Thus, despite similar symptoms and clinical characteristics to those observed in EA there was no evidence of overt airway inflammation in NEA.

Fifty four percent of asthmatic children had no raised sputum eosinophils and the proportion of asthma cases attributable to eosinophilia (PAR) was only 43%, which is comparable with previous studies in adults2,3 and children.22,23  It is possible that, as previously reported, ICS use may have suppressed airway eosinophils and therefore contributed to the low EA prevalence.23,24 However, given that 68% of the NEA group had not used ICS in the last two weeks, ICS use is unlikely to explain these findings. Indeed, this suggests that a substantial proportion of childhood asthma is not associated with appreciable airway eosinophilia.

Although neutrophilic bronchitis is associated with viral exacerbations25 and ICS use in adults,24 we found no evidence that neutrophilic inflammation is involved in stable childhood NEA with levels of neutrophils being highly comparable between NEA, EA and non-asthmatics To our knowledge, only two studies have shown evidence of neutrophilic airway inflammation in stable childhood asthma, but sputum neutrophil percentages (median levels 8.3% 15 and18%14)were considerably lower than those reported in healthy reference populations.26 Other studies have also suggested that NA in children is rare.16,17 Taken together, these studies suggest that neutrophilic involvement is less important in childhood asthma compared with adult asthma, where it may be associated with smoking and occupational exposures.

In the absence of granulocytic inflammation, the cause of asthma symptoms in NEA remains unclear. It is possible that eosinophilic inflammation is present at other airway sites,27 or that the cut-off used for eosinophils (>2.5%)21 has misclassified some asthmatics as NEA when they in fact had low-level eosinophilic inflammation. However, this is unlikely given that in 62% of NEA sputum eosinophils were absent. Misclassification of asthma status may also have occurred, as this was determined primarily on the basis of self-reported symptoms. However, this is also unlikely as this approach has been shown to compare well with objective markers of asthma28 and the prevalence of moderate-to-severe symptoms was very similar between EA and NEA. Thus, in a substantial proportion of childhood asthma, symptoms may be caused by alternative *non-inflammatory* mechanisms, which may have neurogenic or structural origins,29 and which may occur in the absence of on-going airway inflammation in asthma.30,31

We observed no significant clinical differences between EA and NEA in agreement with previous reports.13,14 However, as with adults,8, 9 there are also reports showing that eosinophilic inflammation is associated with greater severity and lung function deficits in childhood asthma.10,12 Furthermore, Lee *et al* showed that sputum eosinophils or neutrophils in EA or NEA respectively was associated with increased asthma severity.23 In contrast, we found that granulocytes in either EA or NEA were not associated with asthma severity, and-moderate-to-severe symptoms had similar prevalence in both phenotypes. We did, however, observe a non-significant trend towards increased symptoms severity in the EA group.

We found a significant inverse association between eosinophils and FEV1-%-predicted, but no associations with other lung function parameters were observed. Intriguingly, we also observed a positive (non-significant) association between neutrophils and FEV1-% -predicted and FEV1/FVC-% -predicted. The mechanisms for these associations are unclear. A possible explanation for the lack of a clear association between lung function and airway inflammation is that lung function in asthmatic children is less strongly associated with symptoms than in adults.32

Unsurprisingly, increased sputum ECP was detected in EA and correlated with eosinophils, as shown previously.32 Similarly, NA and MGA were associated (not statistically significant) with increased levels of NE and IL-8 (Table S1) as previously described.3 Finally, no differences in sputum bacterial endotoxin levels were observed between the groups studied, in contrast with previous findings of increased endotoxin levels in adult NA.6 Sputum endotoxin levels in children were >100 fold lower than in adults. The higher levels in adults may represent increased airway bacterial colonisation during natural ageing.33

A limitation of this study was that only one test per participant was conducted. Some repeated sputum induction studies in adults indicated that NEA is reproducible. 3,34 In contrast, other studies, including two in children, suggest that inflammatory phenotypes are prone to temporal variability,16,17 with some showing a role for ICS treatment in phenotype changes.24 Despite these findings, NEA has been demonstrated in patients with and without ICS treatment, and eosinophilic airway inflammation can persist in patients with ICS-controlled asthma,12 suggesting that EA and NEA can occur independent of ICS treatment. Also, as noted above, in our study 68% of NEA had not used ICS in the last two weeks suggesting that ICS use is unlikely to explain our findings. Nonetheless, we cannot exclude that temporal changes may have affected our results.

In summary, NEA represented approximately 50% of childhood asthma and was not associated with neutrophilic inflammation or other markers of inflammation. Therefore, targeting therapeutic interventions solely towards *inflammation* in NEA may therefore not be effective in a large proportion of asthmatic children; indeed it is possible that absence of inflammation may underlie the poor response to ICS previously observed in >30% of asthmatics.35 Our study adds to previous data3,7 challenging the paradigm that asthma is universally an inflammatory disorder.1 Further research on mechanisms (and environmental exposures) underlying NEA is necessary to guide the development of more effective therapies (and control measures) for NEA, which makes up a considerable proportion of childhood asthma.

**Acknowledgements**

The Centre for Public Health Research is funded by a Programme Grant from the Health Research Council (HRC) of New Zealand, and this research was funded by an HRC Project Grant.

**Figure Legends**

**FIGURE 1.** Sputum eosinophil percentages in (A) asthmatic and non-asthmatic children and (B) non-asthmatic, non-eosinophilic asthma and eosinophilic asthma (Expressed as median (25th-75th centile)) (\*\*\* p < 0.0001)

**FIGURE 2.** Sputum neutrophil percentages in (A) asthmatic and non-asthmatic children and (B) non-asthmatic, non-eosinophilic asthma and eosinophilic asthma (Expressed as median (25th-75th centile)) (\*\*\* p < 0.0001)

**REFERENCES**

1. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008;8(3):183-92.

2. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002;57(7):643-8.

3. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;11(1):54-61.

4. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma : evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest* 2001;119(5):1329-36.

5. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;57(10):875-9.

6. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax* 2007;62(3):211-8.

7. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007;62(12):1043-9.

8. 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(5):753-8.

9. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360(9347):1715-21.

10. 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(2):116-21.

11. Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AW, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998;158(1):36-41.

12. Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. *Eur Respir J* 1998;11(4):848-53.

13. Baraldo S, Turato G, Bazzan E, Ballarin A, Damin M, Balestro E, et al. Noneosinophilic asthma in children: relation with airway remodelling. *Eur Respir J* 2011;38(3):575-83.

14. 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(11):1597-601.

15. 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(9):747-50.

16. 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(3):567-74.

17. Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax* 2012;67(8):675-81.

18. Ellwood P, Asher MI, Beasley R, Clayton TO, Stewart AW. The international study of asthma and allergies in childhood (ISAAC): phase three rationale and methods. *Int J Tuberc Lung Dis* 2005;9(1):10-6.

19. GINA. *Global Strategy for Asthma Management and Prevention 2008 (Update). NHLBI/WHO Workshop Report. Global Initiatives for Asthma*. Washington, D.C.: NHLBI, 2008.

20. ATS. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;152(3):1107-36.

21. 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(5):1008-15.

22. Lovett CJ, Whitehead BF, Gibson PG. Eosinophilic airway inflammation and the prognosis of childhood asthma. *Clin Exp Allergy* 2007;37(11):1594-601.

23. Lee YJ, Kim KW, Choi BS, Sohn MH, Kim KE. Clinical characteristics of eosinophilic and noneosinophilic asthma in children. *Acta Paediatr* 2013;102(1):53-7.

24. Hancox RJ, Cowan DC, Aldridge RE, Cowan JO, Palmay R, Williamson A, et al. Asthma phenotypes: consistency of classification using induced sputum. *Respirology* 2012;17(3):461-6.

25. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J* 2002;19(1):68-75.

26. Balbi B, Pignatti P, Corradi M, Baiardi P, Bianchi L, Brunetti G, et al. Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: values in healthy adults. *Eur Respir J* 2007;30(4):769-81.

27. 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(12):1286-91.

28. Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J* 1999;14(4):951-7.

29. Baroffio M, Barisione G, Crimi E, Brusasco V. Noninflammatory mechanisms of airway hyper-responsiveness in bronchial asthma: an overview. *Ther Adv Respir Dis* 2009;3(4):163-74.

30. Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, et al. Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 2011;364(21):2006-15.

31. Miller BD, Wood BL, Lim J, Ballow M, Hsu C. Depressed children with asthma evidence increased airway resistance: "vagal bias" as a mechanism? *J Allergy Clin Immunol* 2009;124(1):66-73 e1-10.

32. Papadopouli E, Tzanakis N, Tsoumakidou M, Kyriakoy D, Plataki M, Mantzouranis EC, et al. Comparison of induced sputum inflammatory profiles between childhood and adult-onset asthma. *Respir Med* 2006;100(8):1442-50.

33. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Curr Op Immunol* 2010;22(4):507-13.

34. Simpson JL, McElduff P, Gibson PG. Assessment and reproducibility of non-eosinophilic asthma using induced sputum. *Respiration* 2010;79(2):147-51.

35. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002;109(3):410-8.

|  |  |  |
| --- | --- | --- |
| **TABLE 1.** Characteristics of study population. (\* P<0.05; \*\* P<0.01) | | |
|  | **No asthma**  **N=68** | **Asthma**  **N=77** |
| Age (SD) | 15.0 (1.04) | 15.1 (1.01) |
| Males | 72.1% | 76.6% |
| Ethnicity  European  Non-European | 75.0%  25.0% | 68.8%  31.2% |
| Lung function  FVC %-predicted (Mean, SD)  FEV1 %-predicted (Mean, SD)  FEV1/FVC %-predicted (Mean, SD) | 101.1% (12.0)  100.9% (11.4)  100.2% (7.6) | 102.3% (12.8)  96.4% (11.9)\*  94.4% (7.9)\*\* |
| Atopya | 36.8% | 80.5%\*\* |
| Airway hyperreactivityb | 7.6% | 49.4%\*\* |
| Asthma severity  Mild  Moderate  Severe | -  -  - | 19.4%  40.3%  40.3% |
| Asthma persistence  Intermittent  Persistent | -  - | 53.2%  46.8% |

a positive SPT against one or more common allergens

b ≥15% drop in FEV1 from baseline following a 4.5% saline challenge

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **TABLE 2.** Markers of inflammation and endotoxin levels in sputum. Expressed as median (25th-75th centile). \* P<0.05; \*\* P<0.01 asthmatics versus the reference population ,† P<0.05; †† P<0.01 non-eosinophilic versus eosinophilic asthma | | | | |
|  | **No asthma**  **N=68** | **All asthma**  **N=77** | **Eosinophilic asthma**  **N=35** | **Non-eosinophilic asthma**  **N=42** |
| Total eosinophils (x 104/ml) | 0.0 (0.0-3.8) | 28.1 (0.0-228.7)\*\* | 229.9 (89.3-385.7)\*\* | 0.0 (0.0-6.8) †† |
| Total neutrophils (x 104/ml) | 262.4 (49.0-716.0) | 216 (40.6-941.8) | 242.6 (74.0-1233.7) | 170.1 (28.8-856.4) |
| ECP (ng/ml) | 9.0 (2.1-34.3) | 44.6 (9.6-193.8)\*\* | 168.1 (70.3-231.9)\*\* | 12.1 (3.4-49.8) †† |
| NE (µg/ml) | 43.8 (43.8-337.2) | 43.8 (43.8-193.8) | 43.8 (43.8-43.8) | 43.8 (43.8-316.6) |
| IL-8 (ng/ml) | 117.8 (36.1-342.0) | 190.5 (52.1-715.9) | 295 (94.8-1008.8)\* | 163 (46.9-466.3) |
| Endotoxin (EU/ml) | 1.4 (0.7-2.8) | 1.2 (0.6-3.0) | 1.4 (0.6-3.0) | 1.1 (0.5-2.3) |
| Squamous cells (%) | 7.6 (4.1-23.0) | 13.9 (3.4-26.5) | 5.9 (1.4-14.8) | 22.9 (6.1-31.2)†† |
| Columnar epithelials (%) | 0.0 (0.0-0.0) | 0.2 (0.0-0.7)\*\* | 0.2 (0.0-1.0) | 0.2 (0.0-0.6) |

|  |  |  |  |
| --- | --- | --- | --- |
| **TABLE 3.** Differences in clinical characteristics between eosinophilic and non-eosinophilic asthmatics. Analyses were adjusted for age, sex and ethnicity.\* P<0.05; \*\* P<0.01; a positive SPT against one or more common allergens. b ≥15% drop in FEV1 from baseline following a 4.5% saline challenge. c ORs were undefined because either all or none of the subjects in the EA group were positive for the specific symptom or condition | | | |
|  | **Eosinophilic asthmatics (EA), N=35** | **Non-eosinophilic asthmatics (NEA), N=42** | **NEA versus EA**  **N=77** |
|  | *Mean (SD)* | *Mean (SD)* | *∆ %-predicted (95% CI)* |
| Lung function  FVC %-predicted  FEV1 %-predicted  FEV1/FVC %-predicted | 102.0 (14.1)  93.8 (10.6)  92.5 (7.3) | 102.5 (11.8)  98.6 (12.5)  96.1 (8.1) | 0.5 (-5.4 – 6.4)  4.8 (-0.5 – 10.1)  3.6 (0.01 – 7.1)\* |
|  | *%* | *%* | *OR (95% CI)* |
| Atopya | 100.0 | 64.3 | undefinedc |
| Airway hyperreactivityb | 57.1 | 42.9 | 0.5 (0.2 - 1.2) |
| Asthma medication  No asthma medication in last 12 months  ICS in the last 12 months  ICS in the last 2 weeks  β2-agonist in the last 12 months  β2-agonist ≥ once a day in last 2 weeks | 0.0  74.3  48.6  100.0  65.7 | 23.8  54.8  31.0  69.1  42.9 | undefinedc  0.4 (0.1 - 1.2)  0.6 (0.2 - 1.5)  undefinedc  0.4 (0.2 - 1.1 |
| Wheezing attacks in the last 12 months  0-3 times  4-12 times  >12 times | 60.0  31.4  8.6 | 66.7  16.7  16.7 | 1.7 (0.6 - 4.6)  0.4 (0.1 - 1.4)  1.3 (0.3 - 6.0 |
| Sleep disturbance due to wheeze in last 12 months (nights/week)  Never  < than once a week  ≥ once a week | 54.3  40.0  5.7 | 66.7  31.0  2.4 | 2.1 (0.8 – 5.6)  0.6 (0.2 – 1.7)  0.4 (0.0 – 5.2) |
| Speech limited by wheeze in last 12 months | 28.6 | 19.1 | 0.5 (0.2 - 1.6) |
| Asthma severity  Mild  Moderate  Severe | 17.1  34.3  48.6 | 21.4  45.2  33.3 | 1.7 (0.5 – 6.1)  1.7 (0.6 – 4.7)  0.4 (0.2 – 1.2) |
| Asthma persistence  Intermittent  Persistent | 48.6  51.4 | 57.1  42.9 | 1.5 (0.6 – 3.9)  0.7 (0.3 – 1.8) |

a positive SPT against one or more common allergens

b ≥15% drop in FEV1 from baseline following a 4.5% saline challenge

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **TABLE 4:** Associations between cell, cytokine, and LPS levels in sputum and clinical characteristics. Analyses were adjusted for age, sex and ethnicity. | | | | | | | | |
|  | % Eosinophils | Total Eosinophils | % Neutrophils | Total Neutrophils | ECP (ng/ml) | Neutrophil elastase (µg/ml) | IL-8 (ng/ml) | LPS (EU/ml) |
|  | *Regression coefficients (95% CI)c* | | | | | | | |
| FVC %-predicted | -0.27  (-1.42 - 0.89) | -0.05  (-0.16 - 0.06) | 0.53  (0.13 – 0.92)\*\* | 0.00  (-0.00 – 0.01) | 0.28  (-1.11 – 1.66) | 0.03  (-0.00 – 0.07) | 0.09  (-0.00 – 0.18) | -0.14  (-0.34 – 0.07) |
| FEV1 %-predicted | -0.56  (-1.64 – 0.51) | -0.10  (-0.21 -- 0.01)\* | 0.45  (0.08 – 0.83)\* | 0.00  (-0.00 – 0.01) | -0.55  (-1.85 – 0.75) | -0.00  (-0.04 – 0.03) | 0.04  (-0.05 – 0.12) | -0.12  (-0.31 – 0.08) |
| FEV1/FVC %-predicted | -0.25  (-0.98 – 0.48) | -0.05  (-0.12 – 0.03) | -0.03  (-0.29 – 0.23) | 0.00  (-0.01 – 0.01) | -0.76  (-1.64 - 0.12) | -0.03  (-0.06 - -0.01)\* | -0.04  (-0.10 – 0.02) | 0.02  (-0.11 – 0.16) |
|  | *OR (95% CI)d* | | | | | | | |
| Atopya | 31.74  (2.55 – 394.35)\*\* | 1.18  (1.01 – 1.36)\* | 0.95  (0.88-1.03) | 1.00  (1.00 – 1.00) | 1.60  (0.97 – 2.63) | 1.00  (1.00 – 1.01) | 1.00  (1.00 – 1.02) | 1.03  (0.95 – 1.11) |
| Airway hyperreactivityb | 1.25  (0.99 – 1.58) | 0.99  (0.97 – 1.02) | 0.98  (0.90 – 1.06) | 1.00  (1.00 – 1.00) | 1.12  (0.88– 1.43) | 1.00  (1.00 – 1.00) | 1.00  (0.96 – 1.01) | 0.99  (0.95 – 1.03) |

\* P<0.05; \*\* P<0.01. a positive SPT against one or more common allergens

b ≥15% drop in FEV1 from baseline following a 4.5% saline challenge

c Decrease/Increase in % predicted lung function per 5% cells, 10 units of total cells, 100 units of ECP and IL-8, 10 units of NE, or one unit of LPS

d Decrease/Increase in risk per 5% cells, 10 units of total cells, 100 units of ECP and IL-8, 10 units of NE, or one unit of LPS