BRIEF COMMUNICATION

Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma

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Abstract

Background: The inflammatory phenotypes of severe asthma in adults may be reflected in peripheral blood. If this were true in children with severe therapy-resistant asthma (STRA), invasive tests could be avoided. At the moment there is no conclusive evidence in children.
Methods: All patients underwent blood tests, exhaled nitric oxide (FeNO), sputum induction, bronchoalveolar lavage (BAL) and endobronchial biopsy (EB).
Results: Sixty-three (71.6%) patients had a normal blood profile and only 1/88 had a combined blood eosinophilia and neutrophilia. 76/88 (86%) had normal blood eosinophils, but of these, 84% had airway eosinophilia in either BAL ($n = 43;66\%$) or EB ($n = 41;79\%$). In children with STRA blood eosinophilia was associated with airway eosinophilia. However, normal blood eosinophil levels did not exclude airway eosinophilic inflammation.
Conclusions: Peripheral blood counts are not reliable in characterising airway inflammation in severe asthmatic children exposed to high dose steroid therapy, therefore bronchoscopy with BAL should be considered.

Severe asthma in children is characterised by airway eosinophilia (1). The management of children with severe, therapy-resistant asthma (STRA) is proposed to be best determined by inflammatory phenotype (2). Although there is currently no conclusive evidence in children that measurement of inflammation leads to better management, it seems illogical to treat a symptomatic child without airway inflammation with increasingly potent anti-inflammatory medications (3). Inflammatory phenotype is most accurately characterised by bronchoalveolar lavage (BAL) and endobronchial biopsy (EB), obtained at fibreoptic bronchoscopy. Adult data suggest that blood inflammatory profile may correlate with asthma phenotypes (4,5). If this was true in children with STRA, invasive tests could be avoided, and longitudinal measures of systemic inflammation could be used to assess response to therapy.

We hypothesised that differential white blood cell count would be related to pulmonary inflammation in children with

STRA and assessed the association between blood eosinophil count and airway inflammation, measured using exhaled nitric oxide (FeNO), induced sputum, BAL and EB, in STRA children.

Methods

Subjects

Children with STRA aged 6–17 years diagnosed between January 2006 and May 2012 at the Royal Brompton Hospital were recruited. They were part of a bigger group referred for investigation of problematic severe asthma (PSA) (6) defined as the presence of persistent chronic symptoms (≥ 3 days a week), or frequent exacerbations (≥ 1 a month), despite ≥ 800 mcg budesonide, or equivalent daily, a regular long-acting beta agonist, and current or previous failed trial of montelukast (1). To ensure only those with genuine STRA

underwent invasive tests, children with PSA were assessed to confirm diagnosis, exclude significant co-morbidities and to identify underlying modifiable factors that may be contributing to their asthma being problematic (1, 7, 8). In the day of bronchoscopy, a full blood count, including differential white cell count, serum total and allergen-specific IgE (house dust mite, grasses, trees, dog, cat, peanut, milk and egg), spirometry, FeNO and sputum induction were performed. Atopy was defined as ≥ 1 positive specific IgE RAST (≥ 0.34 kU/L) or ≥ 1 positive skin prick test. Patients with STRA underwent fibreoptic bronchoscopy with BAL and EB on the same day (6, 9). The study was approved by The Royal Brompton & Harefield NHLI Ethics Committee.

Spirometry and bronchodilator reversibility (BDR)

Spirometry was performed using a Compact Vitalograph 2120 (Vitalograph, UK) before and 15 min after administration of 1 mg salbutamol via a large volume spacer (10). BDR was defined as an increase of $\geq 12\%$ in $FEV_{1\%}$ predicted.

FeNO

FeNO was measured using the NIOX chemiluminescence analyser (Aerocrine AB, Solna, Sweden) at a flow rate of 50 ml/s (11).

Sputum induction and processing

Sputum induction was performed with 3.5% saline and processed as previously described (12). Normal sputum eosinophil percentage was defined as <2.5% (13) and neutrophils as $\leq 54\%$ (12).

Blood inflammation

Blood inflammatory patterns were defined from peripheral eosinophil and neutrophil counts (4), as neutrophilia >7 $\times 10^9$ neutrophils/L for children aged 6–12 years and >8 $\times 10^9$ neutrophils/L for those >12 years (NEU^H), eosinophilia $\geq 0.1 \times 10^9$ eosinophils/L for all ages (EOS^H) (14).

Bronchoscopy, bronchoalveolar lavage and endobronchial biopsy

FOB, BAL and EB were performed and the samples processed as previously described (15–17). BAL eosinophilia was defined as >1.19% (15), and neutrophilia >3.5% of the total cell count (1, 17).

Statistical analysis

The Mann–Whitney U-test was used to detect differences between groups.

$P < 0.05$ was considered statistically significant. Data were analysed using 'Statistica release 8' (StatSoft Corp., Tulsa, OK, U.S.A.) and 'Stata release 11' (Stata Corporation, TX, U.S.A.).

Results

Subjects

The demographic characteristics of the children are summarised in Table 1.

Blood eosinophil and neutrophil patterns

Blood eosinophil and neutrophil patterns are summarised in Table 1. Only one child had blood eosinophilia and neutrophilia, and 63/88 had a noninflamed blood profile. There was no difference between blood eosinophilic and noneosinophilic patterns in relation to gender, age at referral, passive smoking exposure, family history of asthma, total IgE levels, FEV_1 and FeNO (Table 1). Similarly no difference was found when children were classified according to their blood neutrophil pattern (data not shown). No relationship was found between blood eosinophils and FeNO. Patients on regular systemic corticosteroids had significantly lower blood eosinophils (median, 0.3×10^9/L; IQR, 0.1–0.6) than those not on oral steroids (median, 0.5×10^9/L; IQR, 0.2–0.8), ($P = 0.04$). Positive BAL bacterial culture was associated with elevated blood (median, 5.5[IQR4.3–9.0] vs 3.8[IQR3.0 –4.8]$\times 10^9$/L, $P = 0.002$), BAL (4.3[IQR2.6–28.8] vs 3.1[IQR1.0– 4.7]%, $P = 0.02$) and biopsy (19.7[IQR8.2–47.5] vs 3.8[IQR0.0– 25.5]cells/mm^2, $P = 0.05$) neutrophils.

Blood eosinophils and airway eosinophilia

Although FOB was performed in all patients, not all samples were of sufficient quality for analysis. Sputum, BAL and biopsy inflammatory cells were available from 54, 71 and 57 patients, respectively. Seventy-six (86%) children had a normal blood eosinophil count, but the majority of those (64/76; 84%) had evidence of airway eosinophilia detected in either BAL or EB. When only patients with a normal blood eosinophil count were considered, airway eosinophilic inflammation was present in sputum ($n = 30;68\%$), BAL ($n = 43;66\%$) and EB ($n = 41;79\%$). Of the 12/88 patients with a blood eosinophilic pattern, airway eosinophilia was detected in sputum ($n = 7;55\%$), BAL ($n = 6;100\%$) and EB ($n = 9;89\%$).

Prediction of BAL or biopsy eosinophils from blood eosinophil levels

The positive and negative predictive values of BAL or biopsy eosinophils using the best cut-off for blood eosinophils from a ROC curve from our data are summarised in Table 2.

As the negative predictive values were very low, we also used both BAL and EB eosinophils to determine a score for airway eosinophilia as either normal or abnormal. If this was done, the presence of blood eosinophilia detected airway eosinophilia in all children. However, a normal blood eosinophil count missed airway eosinophilia in 58/76 (76%) cases.

Discussion

The majority of children with STRA (72%) in this study had normal blood eosinophil and neutrophil levels. Critically,

Table 1 Demographic and clinical characteristics of children with STRA according to their blood eosinophilic pattern

Blood inflammatory patterns

EOS Normal/NEU Normal: 63/88 (72%)

EOS Normal/NEU High: 13/88 (15%)

EOS High/NEU Normal: 11/88 (12%)

EOS High/NEU High: 1/88 (1%)

	Noneosinophilic pattern (*n* = 76, 86%)	Eosinophilic pattern (*n* = 12, 14%)	*P* Value
Sex, male (N [%])	42 (55.3)	7 (58.3)	1.00*
Age, year (mean [SD])	12.1 (2.7)	11.9 (2.5)	0.65
Passive smoking (N [%])	18 (23.7)	4 (33.3)	0.47*
Family history of asthma (N [%])	50 (65.8)	8 (66.7)	1.00*
Regular oral steroids (N [%])[†]	32 (42.1)	2 (16.7)	0.12*
Oral steroids, mg/day (median [IQR])	6.37 (5–10)	6.87 (2–12)	0.97
Inhaled corticosteroids, mcg/day (median[IQR])	1200 (800–1600)	1200 (850–1600)	0.51
Total IgE, IU/mL (median [IQR])	434 (144–2230)	498 (113–1110)	0.43
Atopy, (N [%])	60 (78.9)	9 (75.0)	0.69*
FEV1% pred (mean [SD])	72.8 (22.0)	74.5 (19.0)	0.52
FEV1 (Z-score) (mean [SD])	−2.1 (1.8)	−2.0 (1.7)	0.42
FEV1% postbronchodilator (mean [SD])	81.7 (19.8)	82.2 (18.1)	0.38
FEV_1 postbronchodilator (Z-score) (mean [SD])	−1.3 (1.6)	−1.2 (1.5)	0.41
FeNO levels, ppb (median [IQR])	47.0 (25–67)	50.3 (20–77)	0.89

IgE: immunoglobulin E, FEV_1: forced expiratory volume in 1 s, FeNO: fractional exhaled nitric oxide, ppb: parts per billion.
*Fisher's exact test.
†All patients on oral steroids at the time of blood sampling.

Table 2 Prediction of BAL or biopsy eosinophils from blood eosinophil levels

	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV%	NPV%
Blood Eos >0.2 × 10⁹/L				
BAL eosinophilia*	79.6 (65.7–89.7)	68.2 (45.1–86.1)	82	65
Biopsy eosinophilia[†]	76.0 (61.8–86.9)	58.3 (27.8–84.7)	85	33

*AUC(95%CI): 0.81 (0.70–0.89).
†AUC(95%CI): 0.69 (0.56–0.80).

approximately 80% of the children with normal blood eosinophils had evidence of airway eosinophilic inflammation in BAL or EB, which would have been missed if blood inflammation alone had been assessed.

The most important finding in this group of children with STRA, on high-dose steroid therapy, was that 76 (86%) children had no evidence of peripheral eosinophilia, yet approximately two-thirds of those had sputum BAL or biopsy eosinophilia. Thus, airway inflammation would have been missed in a significant majority if blood eosinophil counts alone were used to assess inflammation. However, all twelve of the children with high blood eosinophils also had BAL eosinophilia and nine had biopsy eosinophilia. Our data suggest that if blood eosinophilia is present, it is highly probable that airway eosinophilia (BAL and biopsy) is also present, but if the blood eosinophil count is normal, it is not possible to predict whether airway eosinophilia is present. However,

we cannot determine whether a change in airway eosinophilia would be reflected by a corresponding change in the blood.

We acknowledge that our data only applies to children with STRA. However, this is the first prospective study to report a direct comparison between peripheral blood eosinophilia and airway inflammation in asthmatic children. A previous report in adults has shown a possible use of blood inflammatory indices in defining the clinical heterogeneity of asthma (4). However, that did not include patients with severe disease, did not explore the relationship between blood and airway inflammation and it was a community-based study. A further adult study has shown a relationship between blood and airway inflammation, specifically in neutrophilic disease (5). Although previous studies in children have shown direct correlations between FeNO and blood eosinophils (18), we did not find such a relationship. This is likely because of the influence of high dose inhaled and main-

tenance oral steroid therapy. It is important to note that the results did not change when children on regular oral steroids and those only on high dose inhaled steroids were considered separately (data not shown).

We acknowledge that, even though a recent study in adult refractory asthma has shown the importance of bronchoscopy to identify phenotypes and direct therapy (19), in children, there is no such evidence. At the moment, it is still impossible to determine whether phenotyping asthma using bronchoscopy as part of the initial assessment protocol or making longitudinal measurements of inflammation to guide therapy is clinically beneficial (20). Also, these data are cross-sectional and may be influenced by within-patient phenotypic changes over time, but it is not ethical to perform longitudinal invasive studies in children. Because of ethical limitations, it is difficult to perform serial blood tests in children, and only one sample was taken for clinical indications at the time of bronchoscopy. Recently, Spector et al. (21) showed significant variability in blood eosinophil counts within a 24-h period particularly in moderate asthmatics. We need to acknowledge a potential bias of one time-point measurement in our data; however, our blood sampling was undertaken at approximately the same time of the day (between 2 and 4 pm) and all our patients are children with STRA.

However, the relationship between airway eosinophilia and blood eosinophilia means it will be possible to test the reliability of this relationship in future studies. Finally, that the best cut-off point for blood eosinophils calculated from the ROC curves is very specific for our population of children with severe asthma, in the context of a cross-sectional study.

In summary, we have shown that most children with STRA have a noninflammatory blood profile and that blood indices do not relate to any clinical parameters. Blood eosinophilia suggests that airway eosinophilia is highly likely, but a normal blood eosinophil count does not exclude sputum, BAL or biopsy eosinophilic inflammation. As peripheral blood counts are not reliable in characterising airway inflammation in severe asthmatic children exposed to high dose inhaled and maintenance oral steroid therapy, bronchoscopy with BAL should be considered.

Author contribution

NU was involved in patient recruitment, acquisition and interpretation of data and wrote the manuscript. CB was involved in patient recruitment and acquisition of data. LF contributed to the study design and to the interpretation of data. MS was involved in the analysis of data. AB was involved in the interpretation of data and critical review of the manuscript. SS contributed to the study design, interpretation of data and critical review of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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