Lung Lavage Granulocyte Patterns and Clinical Phenotypes in Children with Severe, Therapy-Resistant Asthma

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Abstract

Background: Children with severe asthma have frequent exacerbations despite guidelines-based treatment with high-dose corticosteroids. The importance of refractory lung inflammation and infectious species as factors contributing to poorly-controlled asthma in children are poorly understood.

Objective: To identify prevalent granulocyte patterns and potential pathogens as targets for revised treatment, 126 children with severe asthma underwent clinically-indicated bronchoscopy.

Methods: Diagnostic tests included BAL for cell count and differential, bacterial and viral studies, spirometry, and measurements of blood eosinophils, total IgE, and allergen-specific IgE. Outcomes were compared among 4 BAL granulocyte patterns.

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Results: Children with pauci-granulocytic BAL were most prevalent (52%), and compared to mixed granulocytic BAL, had less post-bronchodilator (BD) airflow limitation, less blood eosinophilia, and less detection of BAL enterovirus. Children with isolated neutrophilia BAL were differentiated by less blood eosinophilia than mixed granulocytic BAL, but greater prevalence of potential bacterial pathogens compared to pauci-granulocytic BAL. Children with isolated eosinophilia BAL had features similar to mixed granulocytic BAL. Children with mixed granulocytic BAL took more maintenance prednisone, and had greater blood eosinophilia and allergen sensitization compared to pauci-granulocytic BAL.

Conclusions: In children with severe, therapy-resistant asthma, BAL granulocyte patterns and infectious species are associated with novel phenotypic features which can inform pathway-specific revisions in treatment. In 32% of children evaluated, BAL revealed corticosteroid-refractory eosinophilic infiltration amenable to anti-Th2 biological therapies, and in 12%, a treatable bacterial pathogen.

Keywords

Introduction

Most children with asthma treated daily with low- to medium-dose inhaled corticosteroids attain symptom control with few exacerbations [1]. In contrast, children with problematic asthma have frequent symptoms and exacerbations despite treatment with high-dose inhaled and systemic corticosteroids [2–5]. A rigorous approach to the child with problematic asthma includes referral to a specialty center for a staged assessment to confirm the diagnosis, and address remediable factors including co-morbid diagnoses and mitigation of adverse environmental conditions [6–7]. Controller therapies are then adjusted accordingly, and the child is followed by an asthma specialist before a diagnosis of "severe" asthma is made [8]. Those children with frequent symptoms and exacerbations despite these steps have "poorly-controlled, therapy-resistant asthma." However, the extent to which such children have therapy-resistant lung inflammation and detectable lower respiratory infectious species is not well understood. Although sputum, BAL, and blood granulocyte counts have been studied in children with therapy-resistant asthma [9–11], quantitative correlation among those compartments is unreliable, and limits their utility in guiding treatment. Nonetheless, studies of inflammatory markers in BAL and endobronchial biopises have been safely conducted in children with problematic asthma [12]. These investigations demonstrate heterogenous patterns of lung fluid and bronchial wall inflammation, including eosinophilic and neutrophilic infiltration, type 2 innate and diverse helper Th1/Th2/Th17 responses, increased production of reactive chemical species, characteristic BAL cytokine clusters, and impaired macrophage phagocytic function [13–26].

These original reports provide insight into the pattern of lung inflammation in severe asthma of childhood. However, they are based on relatively small samples, with limited information as to whether prevalent inflammatory patterns correspond to specific clinical features. Furthermore, the role of lower respiratory pathogens in informing the lung inflammatory milieu and clinical features in severe asthma has received only limited study [16], despite the

clear importance of infection in the pathobiology of asthma of childhood [27]. Thus a broader understanding of prevalent lung inflammatory patterns and potential infectious species and how they might inform clinical features in children with poorly-controlled, therapy-resistant asthma is necessary. Published guidelines which contain algorithms to advance treatment in childhood asthma are based on studies focused primarily on children with relatively mild asthma [28–29]. Henceforth these algorithms may not be appropriate for children with severe asthma with alternate patterns of inflammation. Therefore, we conducted a prospective study in a well-characterized sample of children referred from community providers with poorly-controlled, therapy-resistant asthma to identify prevalent BAL granulocyte patterns and infectious species and describe the clinical features associated with individual patterns.

Methods

Children with poorly-controlled asthma were referred from a 35-county region in central Virginia to a university-based specialty clinic (Figure 1). These children had an initial assessment (see E-supplement, Methods) based on modification of an evidence-based approach published by the Brompton group [6–7] that included: a) confirmation of the diagnosis of asthma, b) correction of remediable factors, c) evaluation of adherence and co-morbid diagnoses, d) identification of allergen sensitization and appropriate avoidance measures, and e) measurements of lung function with bronchodilator response. After these steps, controller treatment was adjusted according to severity classification [8] and symptom control measures in accordance with standard guidelines [28–29]. Combination high-dose inhaled corticosteroid /long-acting beta agonist therapy was prescribed based on symptom control status, but in some cases denied by the child's payor of care. Children with severe, recurrent exacerbations and poor symptoms were treated with alternate day prednisone 0.5 mg/kg per dose. The children were then followed longitudinally in a specialty clinic by pediatric pulmonologists and allergists. Symptom control was assessed by measures recorded at the first clinic visit and repeated at follow up visits, with poor control defined by a composite index of symptom scores, exacerbations, adherence estimates [30], and lung function results (Figure 1 and E-Supplement).

Children with severe, therapy-resistant, poorly-controlled asthma were offered a diagnostic bronchoscopy with BAL and assessment of blood inflammatory markers. Samples were shared between the clinical and research laboratories through protocols approved by the University of Virginia Institutional Review Board (UVA HSR # 17555, UVA HSR# 10905, and UVA HSR# 10634). Informed consent was provided by the parents or legal guardians, and children > 7 years provided assent. Details of the asthma definition criteria, assessment of remediable factors, adherence, co-morbid diagnoses, exclusion and inclusion criteria, bronchoscopy, BAL, bronchial brushings, and peripheral blood markers are provided in the E- Supplement.

Data Analysis.

The analysis was limited to children age 6 to 17 years with confirmed asthma, demonstrable adherence, with exacerbations and poor control (Figure E1). BAL granulocyte categories as the basis for comparison of phenotypic features were modified from published studies in

adults with asthma [31–33]. Cut points for BAL eosinophilia and neutrophilia (Figure 2) were derived from tables published in the ERS Task Force on BAL in Children [34] and earlier investigations in healthy children [35]. The analytic approach was exploratory as described in the E-Supplement.

Results

2,800 children with poorly-controlled asthma were referred to a regional asthma specialty clinic for assessment and treatment over a 9 year span (Figure 1). Among this sample, 311 children treated with high-dose inhaled and/or systemic corticosteroids with poor symptom control at follow up underwent diagnostic bronchoscopy. 126 of these children, 6 to 17 years of age, with confirmed, therapy-resistant severe asthma are the subject of this report. The features of children who had bronchoscopy but are not included in the analysis are provided in Figure E-1.

General Sample Features

The sample had significant asthma-related morbidity, over 2/3 had been admitted to the hospital in the past year despite treatment with three or more controller medications, with a median daily ICS dose of 800 μg per day fluticasone equivalents (Table E1). The proportion of children treated with daily ICS/LABA was approximately 2/3, lower than expected due to medication costs and non-coverage by the child's payor of care. Co-morbid diagnoses were common, led by gastro-esophageal reflux disease (GERD), obesity, and type IB laryngeal clefts (Table E2).

Features Compared According to BAL Granulocyte Categories

Pauci-Granulocytic.—This was the most prevalent granulocyte category (52%). BAL total cell count was significantly lower in this category compared to the isolated neutrophilia category (Table I). Children with pauci-granulocytic BAL were significantly older than children with BAL isolated neutrophilia, and were less likely to be treated with maintenance prednisone compared to children with isolated eosinophilia or mixed granulocytic BAL (Table II). Children with pauci-granulocytic BAL had greater pre-bronchodilator (pre-BD) $FEV_1\%$, and greater pre-BD FVC% compared to children with isolated eosinophilia (Table III). The proportion of children with post-BD airflow limitation (based on an $FEV_1/FVC <$ 90%) was significantly less than it was in children with mixed granulocytic BAL. Blood eosinophil percentage and absolute blood eosinophil counts were significantly lower in pauci-granulocytic BAL compared to the mixed granulocytic category (Table IV). With regards to detection of potential pathogens in BAL, children in the pauci-granulocytic category had significantly lower prevalence of positive enterovirus/HRV transcripts compared to the mixed granulocytic category (Table V).

Isolated Neutrophilia.—16 % of the children fit the isolated neutrophilia BAL category. Compared to children with pauci-granulocytic BAL, children with isolated neutrophilia had significantly higher BAL total cell counts (Table I), and a lower proportion of non-white minorities and lower median age at bronchoscopy (Table II). Children with isolated neutrophilia had significantly greater pre-BD FEV_1 % compared to isolated eosinophilia,

and higher Pre-BD FEF_{25-75} % compared to mixed granulocytic BAL (Table III). With regards to inflammatory markers, children with isolated neutrophilia had significantly lower blood eosinophil %, lower absolute blood eosinophil counts, and lower blood total IgE values compared to children with mixed granulocytic BAL (Table IV). Detection of any microbe was most prevalent in children with isolated neutrophilia, and specifically detection of potentially pathogenic bacteria was more prevalent in children with isolated neutrophilia compared to children with pauci-granulocytic BAL (Table V).

Isolated Eosinophilia.—9.5% % of children had BAL Isolated eosinophilia. This BAL category was differentiated by the highest prevalence of non-white children (90%), the highest prevalence of hospitalization in the past year (100%), the highest prevalence of past ICU admissions (60%), and the highest prevalence of omalizumab (20%) and antileukotriene treatments among the four categories (p < .05, Fisher's exact test, Table II). The pre-BD FEV_1 % was lower in this category than in children with isolated neutrophilia and pauci-granulocytic BAL, and the pre-BD FVC % was lower than isolated neutrophilia (Table III). Isolated eosinophilia was not associated with significant differences in values of any inflammatory markers or prevalence of potential pathogens.

Mixed Granulocytic.—This was the 2nd most prevalent BAL category, 22% of the total. Children with mixed granulocytic BAL had signficantly lower median BAL macrophages (43%) compared to the pauci-granulocytic category (Table I), and significantly greater prevalence of treatment with maintenance prednisone (Table II). Mean pre-BD FEV_1% was significantly lower in mixed granulocytic BAL compared to isolated neutrophilia (Table III). The prevalence of children with post-BD persistent airflow limitation was significantly higher in mixed granulocytic BAL. The mixed granulocytic category was also differentiated by a relatively higher absolute blood eosinophil count (480 cells/μl; p < 0.05 versus isolated neutrophilia and pauci-granulocytic groups; Table IV), and the highest prevalence of children with peripheral blood eosinophils > 300 cells/μl (p = 0.002). Furthermore children with mixed granulocytic BAL had the highest blood total IgE (322 IU/ml, p < 0.05 versus isolated neutrophilia and pauci-granulocytic groups), and significantly greater sensitization to four or more allergens (58%, p = 0.02). With regard to detection of BAL potential pathogens, children with mixed granulocytic BAL had the highest prevalence of enterovirus/HRV transcripts (Table V).

Correlations Between Inflammatory Markers in Blood and BAL—The % of eosinophils in the blood had a significant (p < 0.001) positive correlation with % of eosinophils in BAL, but the coefficient was low (r = 0.32). To test whether maintenance prednisone treatment might impact the correlation between systemic and lung eosinophils, we did a secondary analysis excluding children treated with oral prednisone. In this secondary analysis the correlation between blood and BAL eosinophil % remained low at 0.36.

ROC curve analysis to depict sensitivity and specificity of the absolute blood eosinophil count as a predictor of BAL eosinophilia was poor, with an area under the curve of 0.66 (Figure E-2). Overall, the sensitivity of an absolute blood eosinophil count > 300 cells/μl blood for BAL eosinophilia was fair, 71% (Table VI), with a positive predictive value of

50%. Likewise the specificity of an absolute blood eosinophil count for BAL eosinophilia was low, 65%, with a negative predictive value of 82%.

Other systemic markers of inflammation, including the total blood neutrophil count and serum C-reactive protein performed poorly as predictors of BAL granulocyte numbers. The sensitivity and specificity of the total blood neutrophil count to predict BAL neutrophilia depicted by ROC curve analysis was not significant with an area under the curve = 0.59. Likewise the serum C-reactive protein performed equally with a low ROC curve area of < 0.60 ROC for both BAL neutrophilia and eosinophilia.

Safety

Bronchoscopy with BAL and bronchial brushing was well tolerated and safe. Post-establishment of general anesthesia, the median time to do a complete examinaton and collect samples was 12 minutes. Minor adverse events included brief laryngospasm (6.5%), wheeze (4.9%), cough (3.3%%), and transient hypoxemia during BAL (1.6%). Two children (1.6%) were admitted electively post-bronchoscopy. No child had a major unexpected adverse event as a result of the bronchoscopy or shared sample procedure. The distribution of individual adverse events was no different according to age category, sex, race, asthma control status, granulocyte pattern, or the presence of pre-BD or post-BD airflow limitation. However obese children had a significantly higher (p = 0.02) prevalence of any adverse event (44%) compared to non-obese children (19%).

The lowest recorded SpO_2 was < 90% in 2 children, 1.5% of the sample. The median SpO_2 upon discharge from the post-anesthesia recovery unit was 98% (97.0–99.0) and no child was discharged with an SpO_2 < 93% in room air. Permissive hypercarbia during emergence from anesthesia was common, 62% of the sample had peak end-tidal CO_2 - values > 45 mm Hg and the highest recorded end-tidal CO_2 was > 60 mm Hg in 14%. Adverse events were no more prevalant in children with permissive hypercarbia > 45 mm Hg compared to those with end-tidal CO_2 < 45 mm Hg.

Discussion

We found that the phenotypic features of children with severe, therapy-resistant asthma are informed by BAL granulocyte categories and detection of potential respiratory pathogens (Figure 2). The two categories with the highest degree of clinical morbidity were mixed granulocytic and isolated eosinophilia, and had in common increased BAL eosinophils despite relatively high prevalance of treatment with systemic prednisone. Although bronchoscopies were postponed in children with symptomatic colds and recent lower respiratory infections, respiratory viruses and/or pathogenic bacteria were detected in 31% of the sample. Our study is novel in so far as most studies of lung fluid or sputum granulocytes in adults and children with severe asthma do not include broad assessment of lower respiratory potential pathogens. Pathogenic bacteria were most prevalent in children with isolated neutrophilia, and rhinovirus/enterovirus transcripts were greatest in the mixed granulocytic category. Children with any respiratory pathogen detected had greater BAL cell counts and neutrophil percentages, were relatively younger, had fewer hospital admissions, shorter duration of asthma, and higher mid-expiratory flow rates (Table E-3). Detection of

pathogenic bacteria could represent colonization or indolent survival in biofilms, and likewise viral detection can correspond to a carrier state, viral replication in the absence of organ involvement, or true infection. Whereas blood granulocyte numbers poorly correlated with their counterparts in BAL, we submit that a diagnostic bronchoscopy is helpful in the care of children with severe, treatment-refractory asthma so as to precisely guide treatment.

We found a considerable number of children adherent to treatment with high-dose corticosteroids had increased BAL and systemic eosinophils. Does this mean these children were corticosteroid-resistant [36]? Glucorticoids decrease lung eosinophilic infiltration through inhibition of epithelial-derived chemotactic cytokines [37], direct induction of eosinophil apoptosis, and they further impede eosinophil production and survival through inhibition of IL-5 [38]. To test whether prednisone treatment might alter the study results, we analyzed the sample features after removing 33 children treated with maintenance prednisone. This did not change in a significant way the distribution of the granulocyte categories nor their salient phenotypic features (Table E-5). Corticosteroid-resistance may occur more frequently than realized in children with asthma. For example in a recent SARP investigation, stable adults and children with asthma given intramuscular triamcinolone had only minor improvements in clinical features and the adults had no significant decrease in sputum eosinophils [39]. Mechanisms of corticosteroid-resistance in asthma are varied and range from impaired drug delivery to the lung per se, glucocorticoid receptor down-regulation, tobacco smoke exposure, and infection [40–43]. Overall second-hand smoke exposure in the sample was not measured directly but is estimated at 46% [44].

This would be the first study to suggest chronic rhinovirus infection may be a factor in poorly-controlled asthma in so far as detection of rhinovirus RNA requires active intracellular replication. *Enteroviradae* transcripts were detected in 36% of children with mixed granulocytic BAL and may have contributed to corticosteroid resistance. Human rhinovirus infection opposes the anti-inflammatory effects of corticosteroids through promotion of eotaxins and mucosal type 2 inflammatory cytokine production including Il-4, IL-5, and IL-13 [45], and disruption of lung epithelial barrier function [46]. The current observations are similar to published observations following hematopoietic stem cell transplants where persistent human rhinovirus type C infection in the lower respiratory tract has been reported [47] and, in the setting of decreased T and NK cell expression, single strains may persist over months [48].

The present study supports a fundamental role of eosinophilic infiltration into the air spaces as an important feature in approximately one-third of children with severe asthma. We would point out that the numbers of children with isolated BAL eosinophilia were relatively few; BAL eosinophilia more often was accompanied by BAL neutrophilia in the sub-group with mixed granulocytic inflammation. In the past decade, an adult-onset "eosinophilic" asthma endotype has been described differentiated by nasal polyps, airflow limitation, and frequent exacerbations [49]. Children with difficult asthma accompanied by corticosteroid-refractory airway mucosal eosinophilia were described by Payne and colleagues over a decade ago [50]. Detection of airway eosinophilic activation best differentiates adults with asthma in complete remission versus those with clinical remission and current asthma [51]. Accordingly, we found that children with BAL eosinophilia with or without neutrophilia had considerable

asthma-related morbidity, despite treatment with maintenance prednisone, and therefore might be considered candidates for anti-eosinophil biological therapies even in the absence of peripheral blood eosinophila. However, although blood eosinophil counts are widely touted as useful markers for initiating biological therapies in asthma, we found blood eosinophils did not reliably predict lung fluid eosinophil counts.

Children with isolated BAL neutrophilia had unique features, with overall less morbidity compared to children with the other granulocyte patterns. They were younger, had more laryngeal clefts, greater detection of BAL bacteria, and relatively higher lung function (Figure 2). Our results are similar to those reported in a sample of children with severe asthma and airway mucosal neutrophilia by Andersson and colleagues [24]. Various mechanisms have been proposed to account for lung neutrophilia in asthma including corticosteroid treatment [36], adipocyte-mediated IL-6 inflammation [52], and respiratory viral [53–55] and bacterial [56-58] infections. Corticosteroid treatment not only does not diminish neutrophilia, but by inhibiting apoptosis, increases the presence of neutrophils and augments neutrophilic inflammation. We did a secondary analysis limited to 20 children with isolated BAL neutrophilia to see if children without BAL potential pathogens had different features from those with pathogens detected. Children with BAL neutrophilia and no detected pathogens were older with longer duration of asthma and trended towards less allergen sensitization than children with BAL neutrophilia and potential pathogens present (Table E-4).

Over one-half the children we studied had pauci-granulocytic BAL with relatively fewer morbid clinical features. This result is different from findings in a previous study of children with severe asthma wherein pauci-granulocytic BAL was found in only 11% [59]. Thirty-six % of adults with asthma in the SARP cohort reported by Hastie et al. had pauci-granulocytic sputum [32]. In an earlier study based on sputum granulocyte categories that included both adults and children with stable asthma, the pauci-granulocytic category was most prevalent regardless of age [60]. Adult asthmatics with pauci-granulocytic sputum had relatively lower IL-5 and IL-13 cytokine levels compared to asthmatics with raised sputum eosinophils [61]. Comparable to our results in children with eosinophilic BAL, a large cohort of adults with asthma and eosinophilic sputum had significantly greater total serum IgE than adults with pauci-granulocytic and neutrophilic sputum [62]. We speculate that children with pauci-granulocytic BAL may represent a sub-group that was originally "Th2 high" [63], but became corticosteroid-resistant, and thus the morbid features we observed in this category were likely driven by non-Th2 and/or non-eosinophilic inflammatory pathways. Thus, children with pauci-granulocytic inflammation already treated with high-dose corticosteroids may be candidates for nonsteroidal therapies and although confirmatory studies are indicated, might even be less responsive to anti-Th-2 biologics including mepolizumab, benralizumab, and dupilumab [64].

The results of our analysis are based on a community-referred sample and thus might be applied to clinical practice. The proportion of children treated with combination ICS/LABA was low in comparison to proportions reported in the European U-BIOPRED cohort (4), but higher than the proportions found in the U.S. SARP III pediatric cohort (5). Thus the study sample has better generalizability for a U.S. compared to a European pediatric severe asthma

population. Hence, a sensitivity sub-analysis was done limited to 88 children who received ICS/LABA treatment (Table E-6). As shown the differentiating features among the granulocyte categories did not change in an important way in the sub-analysis. The granulocyte categories are cross-sectional "snap shots" of a heterogenous inflammatory process, and thus are subject to changes according to treatment, stress, and environmental exposures. In particular constituents of the large conducting airways, which admix with alveolar constituents in BAL, interface closely with the external environment. Thus we found and would expect that the granulocyte patterns are highly prone to variations imposed by environmental exposures including microbes, inhaled irritants, and allergens. BAL granulocyte patterns could have added value in informing adjustments to therapy. Anti-microbials, particularly the macrolides, may have a role in children with lower respiratory bacteria. Children with eosinophilic and mixed granulocytic patterns could be considered for biologicals with the added benefit that these might facilitate a reduction in corticosteroid dosing. Children with isolated neutrophilia in the absence of infection could be treated with novel anti-neutrophil therapies including anti-IL-1β, anti-IL-17, and anti-IL-6. Pauci-granulocytic severe asthma is perhaps the most challenging category, potentially treated with therapies targetting the bronchial epithelium such as macrolides and evolving non-Th-2 novel biologics. Finally, we found important differences in counts of blood and BAL eosinophils, thus assessment of BAL granulocytes via bronchoscopy we suggest could improve selection of biological therapies over utilization of blood alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations

BAL	Bronchoalveolar lavage
BD	bronchodilator
EoE	Eosinophilic esophagitis

FEV$_1$	Forced expired volume in one second
FVC	Forced vital capacity
FEF	Forced expiratory flow
LABA	Long acting beta agonists
ROC	Reciever operating characteristic
RSV	Respiratory syncytial virus
SARP	Severe asthma research program
SpO$_2$	Peripheral capillary oxygen saturation
Th1	T helper one
Th2	T helper two
Th17	T helper seventeen

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Highlights Box

What is already known about this topic?

Severe asthma, despite treatment with high-dose corticosteroids, has varied phenotypic features, but the characteristic patterns of lung granulocytic inflammation and infectious species are not well studied in a community-based setting.

What does the article add to our knowledge?

In children with severe asthma, BAL granulocyte categories and detection of lower respiratory microbes correspond to phenotypic differences in morbid outcomes, airflow limitation, eosinophilia, and degree of allergen sensitization.

How does the study impact currrent management guidelines?

BAL is a safe and effective means to identify corticosteroid-refractory lung eosinophilia and potential bacterial pathogens amenable to revised treatment in children with severe asthma.
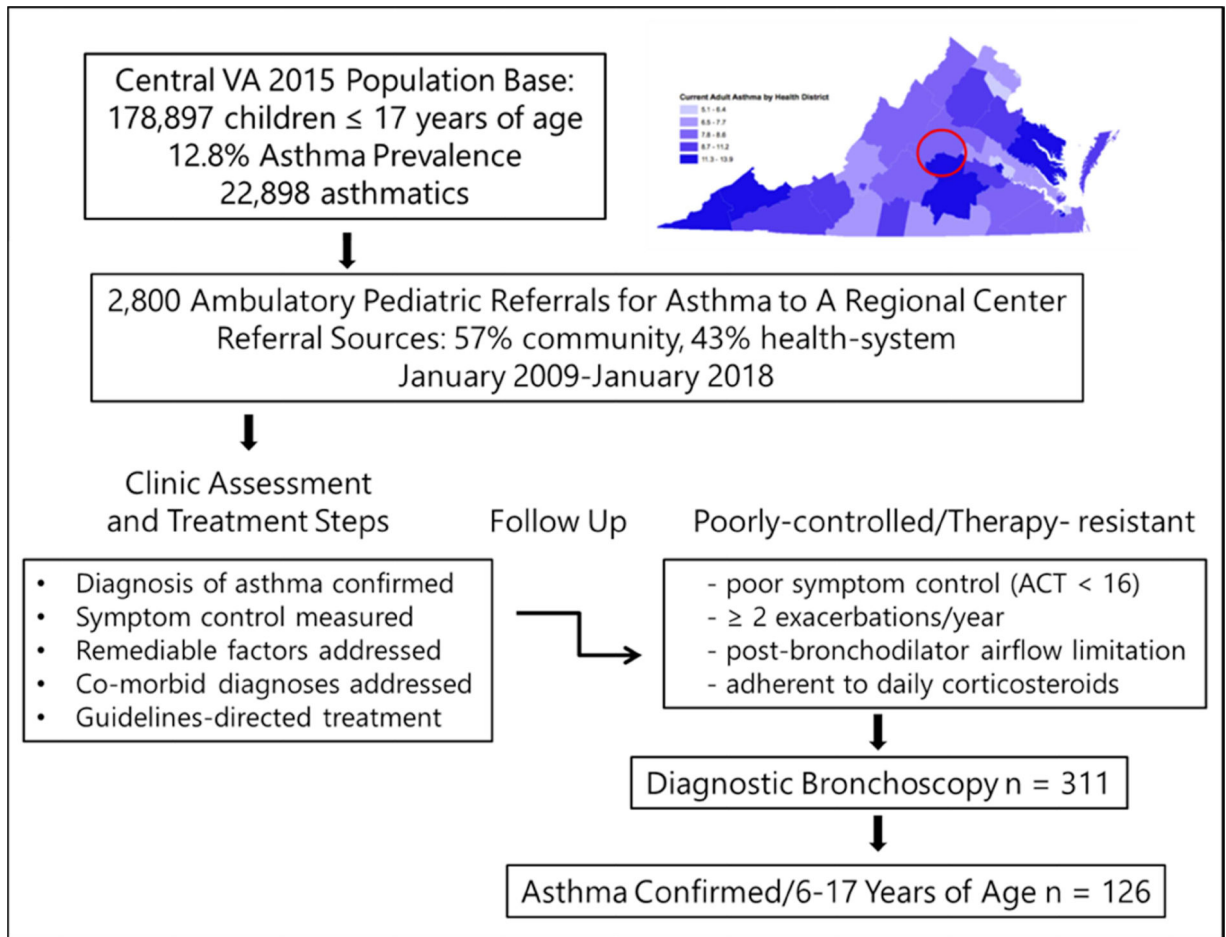
Figure 1.
Consort plot and schematic of the geographic source and clinical assessment, treatment, characterization procedures, and indications for diagnostic bronchoscopy in the study sample.
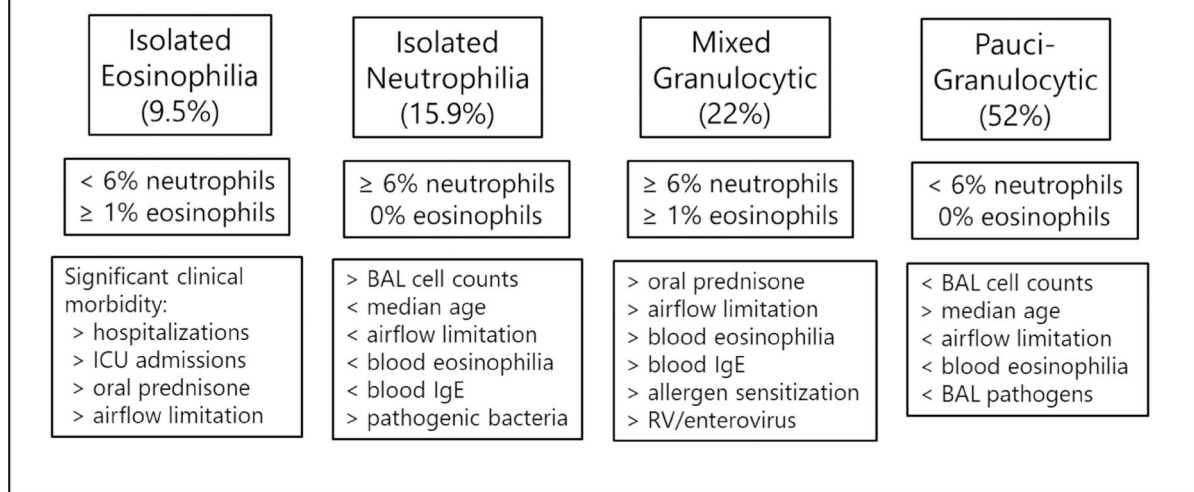
Figure 2.
Prevalent BAL granulocyte patterns and corresponding clinical features

Table I

Prevalent BAL Granulocyte Patterns and Constituents in 126 Children with Poorly-Controlled Asthma

	Isolated Eosinophilia	Isolated Neutrophilia	Mixed Granulocytic	Paucigranulocytic
Sample n (%) [*]	12 (9.5)	20 (15.9)	28 (22.2)	66 (52.4)
Total Cell Count [β] (X 10^6 cells)	1.78 (0.96–2.25)	1.79 [τ] (1.22–5.51)	1.61 (0.57–2.92)	1.08 (0.52–2.20)
Differential Cellular Constituents n (%) [π]				
Macrophages	72 (59–82)	61 (38–82)	43 [a] (15–64)	76 (64–90)
Neutrophils	1 (0–2)	12 (7–38)	23 (11–56)	1 (1–3)
Eosinophils	4 (1–6)	0 (0–0)	3 (2–9)	0 (0–0)
Lymphocytes	3 (2–7	3 (1–6)	3 (1–8)	2 (1–5)
Ciliated epithelial cells	16 (8–30)	10 (4–14)	11 (4–20)	15 (5–28)
Aspiration Marker [θ]				
Lipid-laden macrophage index	0 (0–0)	0 (0–2)	0 (0–1)	0 (0–1)

[*] row percentages

[β] median (25th–75th percentile)

[τ] p = 0.02 vs pauci-granulocytic

[π] expressed as median ± IQR % cells per smear

[a] p < .05 vs pauci-granulocytic

[θ] nominal scale, range 0–4 in intensity.

Table II

Demographic Features, Asthma Severity, and Treatment According to BAL Granulocyte Patterns

	Isolated Eosinophilia n=12	Isolated Neutrophilia n=20	Mixed Granulocytic n=28	Pauci-Granulocytic n=66
Age (years)	10 (8–13)	8 [†] (6–12)	9 (7–12)	12 (8–15)
Male sex n (%)	6 (50)	12 (60)	21 (75)	34 (53)
Non-white n (%)	9 (75) [*]	4 (20)	12 (43)	29 (45)
Body Mass Index (kg/m^2)	19.8 (17.3–22.5)	18.4 (15.6–25.8)	20.6 (15.9–23.9)	21.7 (17.2–27.2)
Asthma Severity Indicators				
Age at Symptom Onset (months)	12 (6–12)	12 (7–24)	12 (12–36)	12 (6–36)
Asthma duration (months)	98 (78–144)	73 (63–102)	78 (57–126)	107 (68–166)
ACT/cACT scores	13 ± 3	16 ± 8	13 ± 7	16 ± 5
Hospitalized in past year n (%)	12 (100) [•]	10 (50)	21 (75)	46 (70)
ICU admission n (%)	6 (60)	4 (21)	8 (29)	19 (30)
Treatment				
Number of daily controller meds.	4 [u] (3–4)	2 (2–3)	2 (1–4)	3 (1–3)
Daily ICS dose (µg fluticasone equiv.)	800 (160–800)	400 (227–800)	800 (400–920)	800 (320–800)
Prednisone Rx n (%)	5 (42)	2 (10)	12 (43)	14 (22)
Omalizumab Rx n (%)	2 (16) [κ]	1 (5)	3 (11)	2 (3)
Mepolizumab Rx n (%)	2 (16)	0	2 (7)	1 (2)
ICS/LABA Rx n (%)	7 (58)	13 (65)	15 (54)	41 (62)
Anti-leuk. n (%)	10 (83) [θ]	9 (45)	11 (39)	41 (62)

Scaled results are median (25th–75th %ile)

[†] p = 0.02 versus pauci-granulocytic

[*] p =0.01

[•] p= 0.04

[u] p = 0.03 versus isolated neutrophilia

p = 0.01

[κ] p = 0.02

[θ] p = 0.004

Table III

Pre- and Post-Bronchodilator Spirometry According to BAL Granulocyte Pattern

	Isolated Eosinophilia n=12	Isolated Neutrophilia n=20	Mixed Granulocytic n=28	Pauci-Granulocytic n=66
Pre-BD FEV_1 % [*]	70 ± 21 [†]	98 ± 19	79 ± 24 [a]	91 ± 18
Post-BD FEV_1 %	82 ± 20	103 ± 19	89 ± 20	98 ± 24
FEV_1 BD % change	25 ± 14	7 ± 10	17 ± 23	12 ± 11
Pre-BD FVC %	81 ± 14 [u]	102 ± 19	91 ± 22	101 ± 16
Post-BD FVC %	94 ± 11	105 ± 22	99 ± 18	106 ± 18
FVC BD % change	16 ± 12 [κ]	4 ± 8	10 ± 11 [μ]	3 ± 4
Pre-BD FEV_1/FVC %	84 ± 13	95 ± 12	86 ± 14	89 ± 10
Post-BD FEV_1/FVC %	87 ± 12	96 ± 9	90 ± 11	94 ± 8
Pre-BD FEF_{25-75} %	56 ± 43	93 ± 35	59 ± 34 [π]	73 ± 30
Post-BD FEF_{25-75} %	66 ± 43	100 ± 30	73 ± 30	89 ± 31
Pre-BD airflow limitation (%)	62%	31%	56%	50%
Post-BD airflow limitation (%)	50%	22%	59% [£]	22%

[*] mean± SD

at the pre-bronchoscopy visit

[†] $p < 0.05$ versus isolated neutrophilia and pauci-granulocytic

[a] $p = 0.03$ versus isolated neutrophilia

[u] $p = 0.02$ versus pauci-granulocytic

[κ] $p < 0.05$ versus isolated neutrophilia and pauci-granulocytic

[μ] $p = 0.03$ versus pauci-granulocytic

[π] $p = 0.02$ versus isolated neutrophilia

[£] $p = 0.03$.

Table IV

Blood and Breath Markers of Inflammation According to BAL Granulocyte Pattern

	Isolated Eosinophilia n=12	Isolated Neutrophilia n=20	Mixed Granulocytic n=28	Pauci-Granulocytic n=66
Blood eosinophil % β	7 (0–10)	2 (1–4)	6 * (3–11)	3 (1–8)
Absolute blood eosinophils (cells/ul)	395 (0–662)	170 (90–460)	480 * (300–850)	180 (90–495)
Peripheral blood eosinophilia n (%) π	6 (50)	5 (25)	20 $\dagger$ (71)	24 (37)
Absolute blood neutrophils (cells/ul)	2830 (1760–3930)	3680 (2945–7470)	3370 (2175–5475)	3145 (2110–4387)
Geometric mean total blood IgE (IU/ml)	297 (97–1378)	55 (16–342)	322 a (170–1091)	194 (56–700)
Number + allergenspecific IgE tests (of 16 tested)	4 (0–12)	1 (0–3)	4 (0–11)	3 (0–7)
Proportion with no allergens n (%)	3 (25)	7 (35)	8 (29)	20 (30)
Proportion 4 allergens n (%)	6 (50)	3 (19)	15 γ (54)	28 (42)
Serum C-reactive protein (mg/dl)	0.42 (0.16–0.62)	0.73 (0.19–1.61)	0.39 (0.15—1.69)	0.43 (0.20–1.86)
C-reactive protein 0.25 n (%)	6 (50)	14 (70)	15 (54)	41 (62)
Expired NO (ppb)	51 (24–51)	9 (5–8)	27 (6–27)	14 (6–14)

Results are median and IQR

β % eosinophils reported in blood count

* $p < 0.05$ versus isolated neutrophilia and pauci-granulocytic

π 300 eosinophils per µl blood

$\dagger$ $p = 0.002$

a $p = 0.05$ versus isolated neutrophilia

γ $p = 0.02$.

Table V

Potential Respiratory Pathogens in BAL According to Granulocyte Pattern

	Isolated Eosinophilia n (%)[*]	Isolated Neutrophilia n (%)	Mixed Granulocytic n (%)	Pauci-Granulocytic n (%)
+ Any Microbe n=39 (31%) [**]	1 (8)	13 (65) [†]	15 (54)	10 (15)
+ Any Respiratory Virus n=29 (24%)	0	9 (45)	13 (46) [†]	7 (11)
+ Any Bacteria n=18 (14%)	1 (8)	7 (35) [β]	5 (18)	5 (8)
+ Virus and Bacteria n=8 (6%)	0	3 (15)	3 (11)	2 (3)
Potential Viral Pathogens Identified n (%)				
Enterovirus/human rhinovirus	0	4 (20)	10 (36) [u]	5 (8)
Human metapneumovirus	0	2 (10)	0	1 (2)
Influenza A	0	1 (5)	1 (4)	0
Influenza B	0	0	1 (4)	0
RSV A	0	1 (5)	0	0
RSV B	0	0	1 (4)	0
Parainfluenza 1	0	1 (5)	0	0
Parainfluenza 2	0	0	0	1 (2)
Adenovirus	0	0	0	0
Potential Bacterial Pathogens Identified n (%)				
Streptococcus pneumoniae	0	2 (10)	3 (11)	1 (2)
Moraxella catarrhalis	1 (10)	1 (5)	0	2 (4)
Hemophilus influenzae	0	3 (15)	0	1
Pseudomonas aeruginosa	0	1 (5)	0	0

[*] column %

[**] sample %

[†] $p < 0.001$

[π] n=121 cultures

[β] $p = 0.02$

[μ] $p = 0.004$.

Table VI

Diagnostic Performance of the Absolute Blood Eosinophil Count to Predict BAL Eosinophilia in Children with Poorly-Controlled, Treatment-Resistant Asthma

True Negatives Absolute Blood Eosinophil Count < 300 cells/ul and BAL Eosinophils < 1% N = 54 (43.5% of total)	False Negatives Absolute Blood Eosinophil Count < 300 cells/ul and BAL Eosinophils 1% N = 12 (9.7% of total)
True Positives Absolute Blood Eosinophil Count 300 cells/ul and BAL Eosinophils 1% N = 29 (23.4% of total)	False Positives Absolute Blood Eosinophil Count 300 cells/ul and BAL Eosinophils < 1% N = 29 (23.4% of total)

Performance Indicators of Absolute Blood Eosinophils > 300 cells/ul for BAL Eosinophilia Sensitivity 71% Specificity 65% Positive Predictive Value 50% Negative Predictive Value 82%