

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



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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, bronchoalveolar lavage 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 current management guidelines? Bronchoalveolar lavage 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.

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 is 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 bronchoalveolar lavage (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.

RESULTS: Pauci-granulocytic BAL was the most prevalent granulocyte category (52%), and children with pauci-granulocytic

BAL had less postbronchodilator airflow limitation, less blood eosinophilia, and less detection of BAL enterovirus compared with children with mixed granulocytic BAL. Children with isolated neutrophilia BAL were differentiated by less blood eosinophilia than those with mixed granulocytic BAL, but greater prevalence of potential bacterial pathogens compared with those with pauci-granulocytic BAL. Children with isolated eosinophilia BAL had features similar to those with mixed granulocytic BAL. Children with mixed granulocytic BAL took more maintenance prednisone, and had greater blood eosinophilia and allergen sensitization compared with those with pauci-granulocytic BAL.

CONCLUSIONS: In children with severe, therapy-resistant asthma, BAL granulocyte patterns and infectious species are associated with novel phenotypic features that can inform

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This study was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program (grant no. U10HL109250-07

to W.G.T.) and the National Institute of Allergy and Infectious Diseases (grant no. U01A123337 to L.B.).

Conflicts of interest: W. G. Teague received honoraria payments as a speaker for Genentech/Novartis. P. W. Heymann has received funding to support research devoted to rhinovirus and asthma from the National Institutes of Health (grant no. U01-AI100799) and from Novartis Pharmaceuticals. J. W. Steinke reports royalty payments from the LUVA cell line. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 4, 2018; revised December 29, 2018; accepted for publication December 31, 2018.

Available online January 14, 2019.

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<https://doi.org/10.1016/j.jaip.2018.12.027>

*Abbreviations used**BAL- bronchoalveolar lavage**BD- bronchodilator**FVC- forced vital capacity**ICS- inhaled corticosteroid**ROC- receiver operating characteristic*

pathway-specific revisions in treatment. In 32% of children evaluated, BAL revealed corticosteroid-refractory eosinophilic infiltration amenable to anti- T_H2 biological therapies, and in 12%, a treatable bacterial pathogen. © 2019 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2019;7:1803-12)

Key words: Severe asthma; Bronchoalveolar lavage; Asthma phenotypes

INTRODUCTION

Most children with asthma treated daily with low- to medium-dose inhaled corticosteroids (ICSs) 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 comorbid 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, bronchoalveolar lavage (BAL), and blood granulocyte counts have been studied in children with therapy-resistant asthma,⁹⁻¹¹ quantitative correlation among these compartments is unreliable, and limits their utility in guiding treatment. Nonetheless, studies of inflammatory markers in BAL and endobronchial biopsies have been safely conducted in children with problematic asthma.¹² These investigations demonstrate heterogeneous patterns of lung fluid and bronchial wall inflammation, including eosinophilic and neutrophilic infiltration, type 2 innate and diverse helper $T_H1/T_H2/T_H17$ 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 that 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 with poorly controlled, therapy-resistant asthma referred from community providers 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 this article’s Methods section in the Online Repository at www.jaci-inpractice.org) 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 comorbid diagnoses, (d) identification of allergen sensitization and appropriate avoidance measures, and (e) measurements of lung function with bronchodilator (BD) response. After these steps, controller treatment was adjusted according to severity classification⁸ and symptom control measures in accordance with standard guidelines.^{28,29} Combination high-dose ICS/long-acting β -agonist therapy was prescribed on the basis of symptom control status, but in some cases denied by the child’s payer 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; see this article’s Online Repository at www.jaci-inpractice.org).

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 older than 7 years provided assent. Details of the asthma definition criteria, assessment of remediable factors, adherence, comorbid diagnoses, exclusion and inclusion criteria, bronchoscopy, BAL, bronchial brushings, and peripheral blood markers are provided in this article’s Online Repository at www.jaci-inpractice.org.

Data analysis

The analysis was limited to children aged 6 to 17 years with confirmed asthma, demonstrable adherence, exacerbations, and poor control (see Figure E1 in this article’s Online Repository at www.jaci-inpractice.org). BAL granulocyte categories as the basis for comparison of phenotypic features were modified from published studies in adults with asthma.³¹⁻³³ Cutoff points for BAL eosinophilia and neutrophilia (Figure 2) were derived from tables published

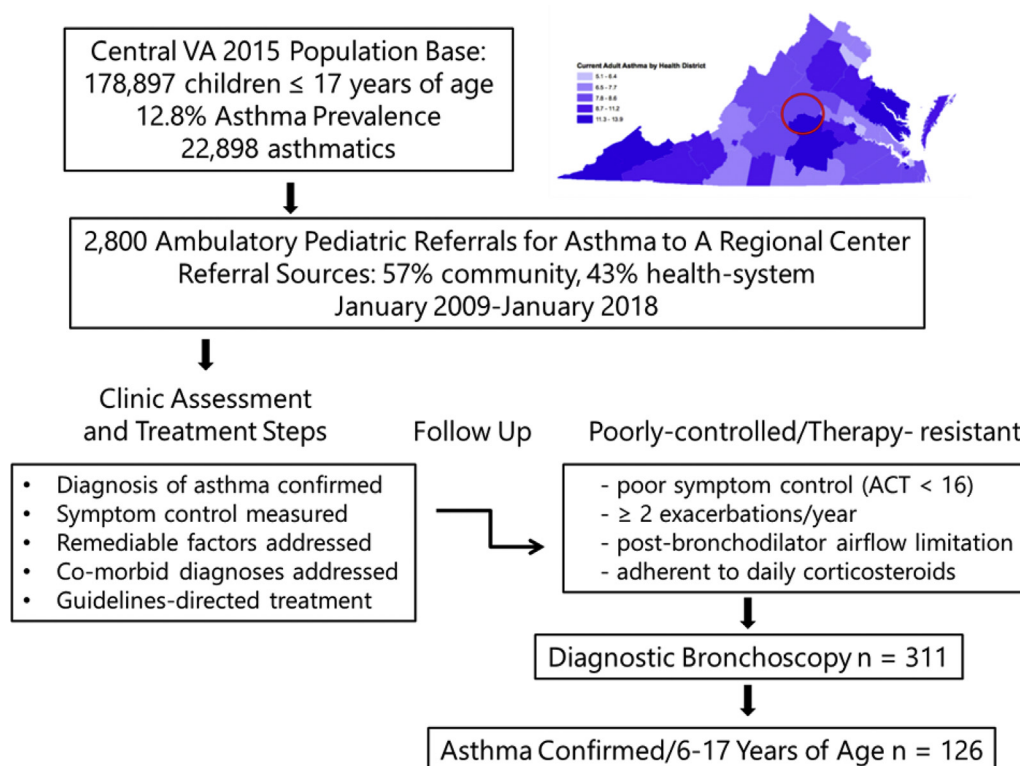


FIGURE 1. Consort plot and schematic of the geographic source and clinical assessment, treatment, characterization procedures, and indications for diagnostic bronchoscopy in the study sample. ACT, Asthma Control Test.

in the ERS Task Force on Bronchoalveolar Lavage in Children³⁴ and earlier investigations in healthy children.³⁵ The analytic approach was exploratory as described in this article's Online Repository at www.jaci-inpractice.org.

RESULTS

A total of 2800 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. A total of 126 of these children, aged 6 to 17 years, 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 E1.

General sample features

The sample had significant asthma-related morbidity; more than two-third had been admitted to the hospital in the past year despite treatment with 3 or more controller medications, with a median daily ICS dose of 800 µg/d fluticasone equivalents (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). The proportion of children treated with daily ICS/long-acting β-agonist was approximately two-third, lower than expected due to medication costs and noncoverage by the child's payer of care. Comorbid diagnoses were common, led by gastro-esophageal reflux disease, obesity, and type IB laryngeal clefts (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

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 than that in 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 with children with isolated eosinophilia or mixed granulocytic BAL (Table II). Children with pauci-granulocytic BAL had greater pre-BD FEV₁ %, and greater pre-BD forced vital capacity (FVC) % compared with children with isolated eosinophilia (Table III). The proportion of children with post-BD airflow limitation (based on an FEV₁/FVC ratio of <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 with the mixed granulocytic category (Table IV). With regard to detection of potential pathogens in BAL, children in the pauci-granulocytic category had significantly lower prevalence of positive enterovirus/human rhinovirus (HRV) transcripts compared with children in the mixed granulocytic category (Table V).

Isolated neutrophilia. Sixteen percent of the children fit the isolated neutrophilia BAL category. Compared with children with pauci-granulocytic BAL, children with isolated neutrophilia had significantly higher BAL total cell counts (Table I), and a lower proportion of nonwhite minorities and lower median age

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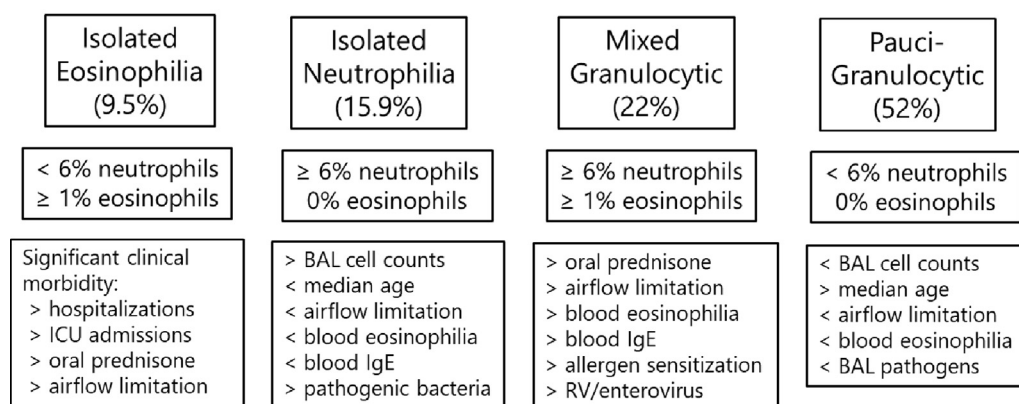


FIGURE 2. Prevalent BAL granulocyte patterns and corresponding clinical features. *ICU*, Intensive care unit; *RV*, rhinovirus.

TABLE I. Prevalent BAL granulocyte patterns and constituents in 126 children with poorly controlled asthma

BAL constituents	Isolated eosinophilia	Isolated neutrophilia	Mixed granulocytic	Pauci-granulocytic
Sample, n (%) [*]	12 (9.5)	20 (15.9)	28 (22.2)	66 (52.4)
Total cell count [†] (× 10 ⁶ cells)	1.78 (0.96-2.25)	1.79 [‡] (1.22-5.51)	1.61 (0.57-2.92)	1.08 (0.52-2.20)
Differential cellular constituents, n (%) [§]				
Macrophages	72 (59-82)	61 (38-82)	43 (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 [¶]				
Lipid-laden macrophage index	0 (0-0)	0 (0-2)	0 (0-1)	0 (0-1)

^{*}Row percentages.

[†]Median (25th-75th percentile).

[‡]*P* = .02 vs pauci-granulocytic.

[§]Expressed as median ± interquartile range % cells per smear.

^{||}*P* < .05 vs pauci-granulocytic.

[¶]Nominal scale, range 0-4 in intensity.

at bronchoscopy (Table II). Children with isolated neutrophilia had significantly greater pre-BD FEV₁ % compared with children with isolated eosinophilia, and higher pre-BD forced expiratory flow at 25% to 75% of FVC % compared with children with mixed granulocytic BAL (Table III). With regard to inflammatory markers, children with isolated neutrophilia had significantly lower blood eosinophil %, lower absolute blood eosinophil counts, and lower blood total IgE values compared with 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 than in children with pauci-granulocytic BAL (Table V).

Isolated eosinophilia. A total of 9.5% of children had BAL isolated eosinophilia. This BAL category was differentiated by the highest prevalence of nonwhite children (90%), the highest prevalence of hospitalization in the past year (100%), the highest prevalence of past intensive care unit admissions (60%), and the

highest prevalence of omalizumab (20%) and antileukotriene treatments among the 4 categories (*P* < .05, Fisher exact test, Table II). The pre-BD FEV₁ % was lower in this category than in children with isolated neutrophilia and pauci-granulocytic BAL, and the pre-BD FVC % was lower than in children with 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 second most prevalent BAL category, 22% of the total. Children with mixed granulocytic BAL had significantly lower median BAL macrophages (43%) compared with children in the pauci-granulocytic category (Table I), and significantly greater prevalence of treatment with maintenance prednisone (Table II). Mean pre-BD FEV₁ % was significantly lower in children with mixed granulocytic BAL than in children with isolated neutrophilia (Table III). The prevalence of post-BD persistent airflow limitation was significantly higher in children in the mixed granulocytic BAL

TABLE II. Demographic features, asthma severity, and treatment according to BAL granulocyte patterns

Sample features	Isolated eosinophilia (n = 12)	Isolated neutrophilia (n = 20)	Mixed granulocytic (n = 28)	Pauci-granulocytic (n = 66)
Characteristic				
Age (y)	10 (8-13)	8* (6-12)	9 (7-12)	12 (8-15)
Male sex, n (%)	6 (50)	12 (60)	21 (75)	34 (53)
Nonwhite, 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 (mo)	12 (6-12)	12 (7-24)	12 (12-36)	12 (6-36)
Asthma duration (mo)	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				
No. of daily controller medicines	4§ (3-4)	2 (2-3)	2 (1-4)	3 (1-3)
Daily ICS dose (µg fluticasone equivalent)	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)¶	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)
Antileukocyte, n (%)	10 (83)#	9 (45)	11 (39)	41 (62)

ACT, Asthma Control Test; cACT, Childhood Asthma Control Test; ICU, intensive care unit; LABA, long-acting β-agonist; Rx, prescription.

Scaled results are median (25th-75th%ile).

**P* = .02 vs pauci-granulocytic.

†*P* = .01.

‡*P* = .04.

§*P* = .03 vs isolated neutrophilia.

||*P* = .01.

¶*P* = .02.

#*P* = .004.

category. The mixed granulocytic category was also differentiated by a relatively higher absolute blood eosinophil count (480 cells/µL; *P* < .05 vs isolated neutrophilia and pauci-granulocytic groups; Table IV), and the highest prevalence of children with peripheral blood eosinophils greater than 300 cells/µL (*P* = .002). Furthermore, children with mixed granulocytic BAL had the highest blood total IgE level (322 IU/mL; *P* < .05 vs isolated neutrophilia and pauci-granulocytic groups), and significantly greater sensitization to 4 or more allergens (58%; *P* = .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 percentage of eosinophils in the blood had a significant (*P* < .001) positive correlation with the percentage 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.

Receiver operating characteristic (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 (see Figure E2 in this article's Online Repository at www.jaci-inpractice.org). Overall, the sensitivity of

an absolute blood eosinophil count greater than 300 cells/µL of 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 of 0.59. Likewise, the serum C-reactive protein performed equally, with a low ROC curve area of less than 0.60 for both BAL neutrophilia and eosinophilia.

Safety

Bronchoscopy with BAL and bronchial brushing was well tolerated and safe. Postestablishment of general anesthesia, the median time to do a complete examination 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 postbronchoscopy. 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

TABLE III. Pre- and post-BD spirometry according to BAL granulocyte pattern

Lung function variables	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‡	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	102 ± 19	91 ± 22	101 ± 16
Post-BD FVC %	94 ± 11	105 ± 22	99 ± 18	106 ± 18
FVC BD % change	16 ± 12¶	4 ± 8	10 ± 11#	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**	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

FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of FVC.

*Mean ± SD.

†P < .05 vs isolated neutrophilia and pauci-granulocytic.

‡P = .03 vs isolated neutrophilia.

§At the prebronchoscopy visit.

||P = .02 vs pauci-granulocytic.

¶P < .05 vs isolated neutrophilia and pauci-granulocytic.

#P = .03 vs pauci-granulocytic.

**P = .02 vs isolated neutrophilia.

††P = .03.

TABLE IV. Blood and breath markers of inflammation according to BAL granulocyte pattern

Inflammatory markers	Isolated eosinophilia (n = 12)	Isolated neutrophilia (n = 20)	Mixed granulocytic (n = 28)	Pauci-granulocytic (n = 66)
Blood eosinophil %*	7 (0-10)	2 (1-4)	6† (3-11)	3 (1-8)
Absolute blood eosinophils (cells/μL)	395 (0-662)	170 (90-460)	480‡ (300-850)	180 (90-495)
Peripheral blood eosinophilia, n (%)‡	6 (50)	5 (25)	20§ (71)	24 (37)
Absolute blood neutrophils (cells/μL)	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 (170-1091)	194 (56-700)
No. of positive allergen-specific IgE test (of 16 tested) results	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 with ≥4 allergens, n (%)	6 (50)	3 (19)	15¶ (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.

*% eosinophils reported in blood cell count.

†P < .05 vs isolated neutrophilia and pauci-granulocytic.

‡≥300 eosinophils/μL of blood.

§P = .002.

||P = .05 vs isolated neutrophilia.

¶P = .02.

had a significantly higher ($P = .02$) prevalence of any adverse event (44%) compared with nonobese children (19%).

The lowest recorded peripheral capillary oxygen saturation was less than 90% in 2 children, 1.5% of the sample. The median peripheral capillary oxygen saturation on discharge from the postanesthesia recovery unit was 98% (97.0%-99.0%), and no child was discharged with a peripheral capillary oxygen saturation of less than 93% in room air. Permissive hypercarbia during emergence from anesthesia was common; 62% of the sample had peak end-tidal CO₂ values of more than 45 mm Hg and the highest recorded end-tidal CO₂ value was more than 60 mm Hg

in 14%. Adverse events were no more prevalent in children with permissive hypercarbia (>45 mm Hg) compared with those with end-tidal CO₂ value of less than 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 2 categories with the highest degree of clinical morbidity were mixed granulocytic and isolated eosinophilia, and

TABLE V. Potential respiratory pathogens in BAL according to granulocyte pattern

Potential pathogens	Isolated eosinophilia, n (%) [*]	Isolated neutrophilia, n (%)	Mixed granulocytic, n (%)	Pauci-granulocytic, n (%)
Respiratory pathogen				
+ 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) [§]	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)	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 (%)				
<i>Streptococcus pneumoniae</i>	0	2 (10)	3 (11)	1 (2)
<i>Moraxella catarrhalis</i>	1 (10)	1 (5)	0	2 (4)
<i>Hemophilus influenzae</i>	0	3 (15)	0	1
<i>Pseudomonas aeruginosa</i>	0	1 (5)	0	0

RSV, Respiratory syncytial virus.

^{*}Column %.

[†]Sample %.

[‡] $P < .001$.

[§] $P = .02$.

^{||} $P = .004$.

had in common increased BAL eosinophils despite relatively high prevalence of treatment with systemic prednisone. Although bronchoscopies were postponed in children with symptomatic colds and recent lower respiratory tract 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 tract 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 midexpiratory flow rates (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). 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. Although 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 that 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 Glucocorticoids decrease lung eosinophilic

infiltration through inhibition of epithelial-derived chemotactic cytokines³⁷ and 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 (see Table E5 in this article's Online Repository at www.jaci-inpractice.org). Corticosteroid resistance may occur more frequently than realized in children with asthma. For example, in a recent Severe Asthma Research Program 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 downregulation, 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 that chronic rhinovirus infection may be a factor in poorly controlled asthma in so far as detection of rhinovirus RNA requires active intracellular replication. *Enteroviridae* 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

TABLE VI. Diagnostic performance of the absolute blood eosinophil count to predict BAL eosinophilia in children with poorly controlled, treatment-resistant asthma

True negatives	False negatives
Absolute blood eosinophil count	Absolute blood eosinophil count
<300 cells/ μ L and BAL eosinophils <1%	<300 cells/ μ L and BAL eosinophils $\geq$ 1%
N = 54 (43.5% of total)	N = 12 (9.7% of total)
True positives	False positives
Absolute blood eosinophil count	Absolute blood eosinophil count
$\geq$ 300 cells/ μ L and BAL Eosinophils $\geq$ 1%	$\geq$ 300 cells/ μ L and BAL eosinophils <1%
N = 29 (23.4% of total)	N = 29 (23.4% of total)
Performance indicators of absolute blood eosinophils	
>300 cells/ μ L for BAL eosinophilia	
Sensitivity 71%	
Specificity 65%	
Positive predictive value 50%	
Negative predictive value 82%	

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 reported⁴⁷ and, in the setting of decreased T- and natural killer-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 number of children with isolated BAL eosinophilia was relatively few; BAL eosinophilia more often was accompanied by BAL neutrophilia in the subgroup 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 et al⁵⁰ over a decade ago. 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 antieosinophil biological therapies even in the absence of peripheral blood eosinophilia. However, although blood eosinophil counts are widely touted as useful markers for initiating biological therapies in asthma, we found that blood eosinophils did not reliably predict lung fluid eosinophil counts.

Children with isolated BAL neutrophilia had unique features, with overall less morbidity compared with 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 et al.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 viral⁵³⁻⁵⁵ and bacterial⁵⁶⁻⁵⁸ 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 whether 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 toward less allergen sensitization than did children with BAL neutrophilia and potential pathogens present (see Table E4 in this article's Online Repository at www.jaci-inpractice.org).

More than 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 percent of adults with asthma in the Severe Asthma Research Program cohort reported by Hastie et al³² had pauci-granulocytic sputum. 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 Adults with asthma with pauci-granulocytic sputum had relatively lower IL-5 and IL-13 cytokine levels compared with those with asthma 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 did adults with pauci-granulocytic and neutrophilic sputum.⁶² We speculate that children with pauci-granulocytic BAL may represent a subgroup that was originally “T_H2 high,”⁶³ but became corticosteroid-resistant, and thus the morbid features we observed in this category were likely driven by non-T_H2 and/or noneosinophilic 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-T_H2 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/long-acting β -agonist was low in comparison to proportions reported in the European U-BIOPRED cohort,⁴ but higher than the proportions found in the US Severe Asthma Research Program III pediatric cohort.⁵ Thus, the study sample has better generalizability for a US compared with a European pediatric severe asthma population. Hence, a sensitivity subanalysis was done limited to 88 children who received ICS/long-acting β -agonist treatment (see Table E6 in this article's Online Repository at www.jaci-inpractice.org). As shown, the differentiating features among the granulocyte categories did not change in an important way in the subanalysis. The granulocyte categories are cross-sectional “snap shots” of a heterogeneous 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. Antimicrobials, particularly the macrolides, may have a role in children with lower respiratory tract 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 antineutrophil therapies including anti-IL-1 β , anti-IL-17, and anti-IL-6. Pauci-granulocytic severe asthma is perhaps the most challenging category, potentially treated with therapies targeting the bronchial epithelium such as macrolides and evolving non-T_H2 novel biologics. Finally, we found important differences in counts of blood and BAL eosinophils. Thus, we suggest that assessment of BAL granulocytes via bronchoscopy could improve selection of biological therapies over utilization of blood alone.

Acknowledgements

We acknowledge the children and family who volunteered to be in this study and shared clinical samples with the research laboratory. We likewise acknowledge the steadfast efforts of the research coordinators, Kristin Wavell, Denise Thompson-Batt, and Theresa Altherr, and laboratory technicians, Kimberly De Ronde and Martha Spanno. The efforts of the anesthesiology attending staff and residents are appreciated, as well as the clinical laboratory managers and staff, and attending pathologists. Administrative staff included Lyn Melton and Wendy Cline, and grants administrators included Angela Rogers and Michelle Haynes.

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ONLINE REPOSITORY

METHODS

Asthma definition and severity classification

The diagnosis of asthma was confirmed according to criteria endorsed by the National Institutes of Health/National Heart, Lung, and Blood Institute EP-3 guidelines^{E1} with procedures followed in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program^{E2}: (1) physician diagnosis, (2) current treatment, (3) characteristic symptom pattern, and (4) documented BD reversibility (12% increase in FEV₁ above baseline postalbuterol) and/or a positive methacholine challenge (PC₂₀ < 16 mg/mL). Enrollees met criteria for severe or very severe asthma based on an adaptation of the European Respiratory Society/American Thoracic Society consensus definition.^{E3} Corticosteroid treatment was expressed in daily fluticasone equivalents and the cutoff point for high-dose corticosteroid treatment defined according to the Global Initiative for Asthma Guidelines^{E4} and Severe Asthma Research Program thresholds.^{E2} High-dose ICSs must be taken for the 3 months before enrollment and for at least 6 of the past 12 months.

Assessment of remediable factors

At the initial assessment visit and at each follow-up clinic visit, remediable factors as recommended by Bush and Saglani^{E5} and Sharples et al^{E6} were assessed. Procedures included a session with a respiratory therapist to review spacer availability, cleanliness, and metered dose inhaler technique. Procedures to cleanse nebulizer masks and delivery tubes were discussed. A registered nurse with additional certification as an Asthma Educator was available at each clinic visit to review medications and renew prescriptions. A second dedicated respiratory therapist was available to discuss scheduled home respiratory therapy practices. Prescription refill status was updated at each clinic visit, and refills could be requested on a call-in basis during each working day. Adherence was checked using a published method.^{E7} School forms, which included a description of an asthma action plan, access to inhaled rescue medications, and authorized use preexercise, were completed for each child. At each clinic visit the attending physician updated the child's asthma action plan, which was printed and handed to the care provider at the end of each session by the physician. Environmental allergies were reviewed at each clinic visit, and specific avoidance measures for dust mite, mold, cat, and dog were reviewed. Because of the geographic distances traveled by our patients, we were unable to conduct home nursing visits.

Assessment and treatment of comorbid and overlap diagnoses

Obesity was defined as body mass index greater than or equal to 85% for age. Obese children were offered nutritional counseling, referred to a child fitness clinic, and encouraged to participate in exercise classes (Zumba) held onsite or at the local neighborhood YMCA. Children with nightly snoring and symptoms of *sleep-disordered breathing* with adenotonsillar enlargement were treated initially with nasal corticosteroids and montelukast. Children with treatment-refractory symptoms had overnight sleep studies, and those with an apnea hypopnea index of more than 5 were referred to otolaryngology for adenotonsillectomy. Children with symptoms of *vocal cord dysfunction* were evaluated initially by spirometry, with inspection of the

inspiratory phase of the flow-volume loop for variable extra-thoracic obstruction pattern before and after BDs, or by treadmill challenge with spirometry. Children with criteria for vocal cord dysfunction based on history or as identified by lung function testing were referred to an otolaryngologist for ambulatory flexible rhinolaryngoscopy and speech therapy. Children with mild vocal cord dysfunction were retained in the data set provided they also met criteria for asthma and underwent treatment. Children with symptoms of *chronic sinus disease* underwent sinus computed tomography scans, and were treated accordingly with antibiotics and nasal corticosteroids. Children with symptoms of *gastro-esophageal reflux* (gastro-esophageal reflux disease) were referred to gastroenterologists and then evaluated by imaging, esophagogastroduodenoscopy (EGD) with esophageal biopsy, and/or 24 esophageal pH monitoring and treated accordingly with proton pump inhibitors. *Intermittent aspiration* was considered likely in children with dysphagia or related conditions and abnormal swallow imaging studies, and positive lipid-laden macrophages were treated with long-term speech therapy and surgical correction of related lesions including microflap closure of laryngeal clefts. *Eosinophilic esophagitis* was managed by a team of pediatric gastroenterologists and allergists, was present in children with allergic food sensitization and a positive esophageal biopsy for epithelial eosinophils, and treated with high-dose proton pump inhibitors, swallowed corticosteroid preparations, and/or mepolizumab. *Premature birth* and estimated gestational age (EGA) were assessed according to maternal recall and confirmed when possible via the medical record. Children with *recurrent sinopulmonary infections* were evaluated for primary ciliary dyskinesia (protocol below) and if indicated had routine sweat chloride measures and assessment of innate and adaptive immunity by a pediatric immunologist. Blood tests included quantification of total immunoglobulin IgG, IgA, and IgM, measures of antidipteria and/or antitetanus serologies, and assessment of specific pneumococcal antibody serologies before and after challenge with pneumococcal vaccine. *Airway structural anomalies* were assessed at bronchoscopy through examination of the larynx and branching airways. Laryngeal clefts were graded according to depth through rigid laryngoscopy and a probe. None of the clefts extended below the plane of the superior cricoid cartilage. *Right middle lobe syndrome* was diagnosed in children with persistent opacification of the right middle lobe on serial radiographics. *Gross ciliary motion* was evaluated in bronchial brush samples removed adjacent to the carina. The brush tips were submitted in fresh medium immediately to the cytology laboratory for examination by a pathologist by video microscopy. Ciliary motion was graded as not present, present, or sample insufficient for analysis. Children with absent ciliary motion and recurrent sinopulmonary infections underwent electronic microscopic studies of ciliary ultrastructure and genetic testing for primary ciliary dyskinesia.

Criteria for exclusion

Children with isolated vocal cord dysfunction without asthma, protracted bacterial bronchitis syndrome without asthma, significant immunodeficiencies, severe aspiration syndromes, primary ciliary dyskinesia, major congenital heart disease, cystic fibrosis, premature birth (<36 weeks estimated gestational age), and major structural airway anomalies were excluded from the data analysis. Children with treated comorbid diagnoses and diagnoses known to overlap or coexist with chronic asthma were

not excluded from the analysis. These disorders included obesity, mildly premature birth (36–38 weeks), treated sleep-disordered breathing, vocal cord dysfunction,^{E8,E9} chronic sinusitis, gastroesophageal reflux disease, treatable and mild intermittent aspiration, eosinophilic esophagitis,^{E10} type I laryngeal clefts,^{E11} mild trachea-broncho-malacia,^{E11} and chronic right middle lobe opacification.^{E12} Children with absent ciliary motion were retained in the data set provided they had no history of recurrent sinopulmonary infections and had normal electron microscopy (EM) and genetic studies.^{E13,E14}

Definitions of therapy-resistant/poorly controlled asthma

Criteria for poorly controlled asthma were (a) Asthma Control Test/Childhood Asthma Control Test (cACT) scores in the 13 to 16 range or lower, (b) 2 or more exacerbations per year requiring systemic corticosteroids, (c) any asthma-related intensive care unit admission in the past year, or (d) persistent air-flow limitation ($FEV_1 < 80\%$ predicted or an FEV_1/FVC ratio of $<90\%$ predicted). Therapy-resistant asthma was present in children with poorly controlled asthma despite at least 3 months of treatment with guidelines-appropriate controller medications, and consistent adherence to controller therapies.

Flexible bronchoscopy procedure

Children presented to the preoperative suite with their parents and were evaluated by an attending pediatric anesthesiologist and bronchoscopist. Children recovering from infections or a major exacerbation in the last 4 weeks or children with nasal discharge on examination did not undergo bronchoscopy but were offered a rescheduled appointment. Children with wheeze on examination but in no distress were given BDs and anxious children midazolam preoperatively. Bronchoscopies were done via laryngeal mask airway under general anesthesia. Up to 4 mg/kg 1% to 2% sterile lidocaine solution was splashed directly onto the vocal cords and carina. Care was taken to introduce the bronchoscope under as “sterile” conditions as possible by avoiding aspiration through the instrument port before doing the BAL. The instrument port of the bronchoscope was routinely flushed with warm sterile saline and potentially contaminated suction traps replaced with sterile units before attempting BAL. The interarytenoid notch was examined for clefts, and the depth of visible clefts was probed by an otolaryngologist. The branching airways were then examined, with the child spontaneously breathing to the level of segmental divisions with careful note of the presence and severity of trachea-malacia or broncho-malacia.

Bronchoalveolar lavage

BAL was done with 2 to 3 flushes of 1 mL/kg (max 40 mL) sterile saline warmed to 37°C, most often in the right middle lobe (88.6%). The lavage return was controlled by gentle sub-atmospheric pressure to avoid mucosal injury and on average was 0.28 of the instilled volume. BAL was submitted fresh to the hospital cytopathology laboratory for cytospin preparations, staining, and manual differential counts. BAL cell granulocyte differentials were further validated by an immunopathologist by hand under a separate protocol number (UVA HSR# 10905).

Smears were examined by a cytopathologist for extracellular or intracellular bacteria and the pattern of inflammation. Smears also underwent an oil red O stain for the number of lipid inclusions in macrophages graded on a scale of 0 to 4. An aliquot of BAL was sent immediately to the Clinical Microbiology and Molecular Diagnostics Laboratory for qualitative bacterial gram stain, culture, and sensitivities and PCR for respiratory viral pathogens (Luminex xTAG reagents, LiquiChip) including adenovirus, human metapneumovirus, influenza A (subtype H1N1, 2009 H1N1), influenza B, parainfluenza 1,2,3,4, respiratory syncytial virus A-B, and *Enteroviridae*/human rhinovirus.

Bronchial brushings

Bronchial epithelial cells for analysis of gross ciliary motion were obtained adjacent to the carina under direct visualization with a cytology brush. The brush tips with epithelial cells were submitted fresh in RPMI for evaluation by a cytopathologist. A standard microscope with a lowered condenser lens produced diffraction to visualize the cilia. Ciliary motion was graded by the cytopathologist as an insufficient sample for analysis, vigorous and synchronized, or absent.

Blood biomarkers

With the child under anesthesia and before the bronchoscopy a sample of venous blood was sent for complete blood cell count and differential including absolute neutrophil and eosinophil counts, total IgE, allergen-specific blood IgE tests, and serum C-reactive protein. The cutoff point for absolute blood eosinophilia was greater than or equal to 300 cells/ μ L of blood.^{E2} Children with allergen sensitization had 1 or more positive skin-prick allergen test result or a specific blood allergen IgE titer of more than 1.5 units out of a panel of 16 common inhalant allergens and 5 common foods.^{E2} “Hypersensitization” was present in children with 4 or more allergen sensitizations.^{E2} Serum C-reactive protein was measured in the hospital clinical laboratory.

Lung function

Spirometry was done at each of the clinic visits and tests done at the prebronchoscopy visit used to report lung function adjusted for age, sex, height, and race/ethnicity based on US population reference standards.^{E15} The cutoff point for airflow limitation was greater than or equal to 1.5 SD below the population reference mean value and an FEV_1/FVC ratio of less than 90% predicted.^{E15} Patients underwent a maximum BD test based on the Severe Asthma Research Program III manual of procedures with a cutoff point of a positive BD response of more than 12% increase above the baseline FEV_1 % predicted.^{E2} Methacholine bronchoprovocation tests were done when indicated to confirm bronchial hyperresponsiveness with the tidal breathing method according to American Thoracic Society standards and the Severe Asthma Research Program manual of procedures.^{E2}

Analysis of results

The distribution of categorical variable count data was tested with Pearson chi-square with Fisher exact test. The relative impact of an individual variable on the chi-square distribution was examined by the absolute value of the standardized residuals. Scaled continuous data were tested for a Gaussian distribution by

the Kolmogorov-Smirnoff calculation. Central dispersion of non-Gaussian data was expressed as the median value and interquartile range (25th-75th percentile) and subgroup comparisons made with the Kruskal Wallis test corrected for multiple comparisons (Bonferroni). Blood IgE values were expressed as geometric means. Central dispersion of Gaussian data was expressed as the mean value and SD and subgroup comparisons made with ANOVA corrected for multiple comparisons (Bonferroni).

Bivariate correlations between blood and BAL markers were tested with Spearman rho. The performance of blood granulocyte counts to accurately depict BAL granulocytes was tested by analysis of receiver operating characteristic curves. We report *P* values of less than .05 to identify probabilities that the differences between granulocyte patterns and specific clinical features were greater than by chance alone. All analyses were done with SPSS version 24 (IBM Corporation, Armonk, NY).

TABLE E1. Phenotypic features of 126 children with poorly controlled, treatment-refractory asthma

Features	Value
Median (IQR) BAL total cell count ($\times 10^6$)	1.36 (0.68-2.70)
Median % BAL macrophages	68 (45-82)
Median % BAL neutrophils	3 (1-11)
Median % BAL eosinophils	0 (0-1.2)
Median % BAL lymphocytes	3 (1-5)
Median % BAL ciliated epithelial cells	13 (5-23)
Median lipid-laden macrophage index (range, 0-4)	0 (0-1)
Demographic/anthropometric features	
Median age (y)	10.6 (7.6-13.9)
Male sex, n (%)	76 (60)
White ethnic/racial background, n (%)	72 (57.1)
Black ethnic/racial background, n (%)	45 (35.7)
Mixed ethnic/racial background, n (%)	9 (7.1)
Median BMI (kg/m^2)	20.3 (16.3-25.1)
% obesity (BMI > 85 th ile)	18 (14.3)
Asthma control and severity indicators	
Mean ACT/cACT scores	15.5 $\pm$ 5.6
% Hospital admission for asthma in past year	89 (70.6)
% with ICU admission for asthma lifetime	37 (29.4)
Median duration of asthma (mo)	98.5 (67.0-141.0)
Treatment	
Median no. of daily controller therapies	3 (2-4)
Median daily ICS dose (μg fluticasone equivalents)	800 (320-800)
% Maintenance prednisone	34 (27.0)
% Any biological	13 (10.3)
% Long-acting BD	77 (61.1)
% Antileukotriene	72 (57.1)
Spirometry	
Mean pre-BD FEV ₁ % predicted	85.9 $\pm$ 22.5
Mean post-BD FEV ₁ % predicted	94.8 $\pm$ 23.0
Mean % change from baseline post-BD FEV ₁	13.9 $\pm$ 15.5
Mean pre-BD FVC % predicted	98.0 $\pm$ 20.3
Mean post-BD FVC % predicted	103.1 $\pm$ 18.5
Mean % change from baseline post-BD FVC	6.2 $\pm$ 9.0
Mean pre-BD FEV ₁ /FVC % predicted	87.1 $\pm$ 12.9
Mean post-BD FEV ₁ /FVC % predicted	92.6 $\pm$ 10.0
Mean pre-BD FEF ₂₅₋₇₅ % predicted	65.8 $\pm$ 32.5
Mean post-BD FEF ₂₅₋₇₅ % predicted	84.3 $\pm$ 33.3
% Pre-BD airflow limitation (n = 104)	53 (51)
% Post-BD airflow limitation (n = 71)	25 (35.2)
Blood and breath markers of inflammation	
Median % blood eosinophils	4 (2-9)
Median absolute blood eosinophil count (no. of cells/ μL)	290 (140-630)
% with absolute blood eosinophils $\geq$ 300 cells/ μL	57 (46.0)
Median absolute blood neutrophil count (cells/ μL)	2940 (2110-4660)
Geometric mean total blood IgE (IU/mL)	190
Median no. of positive blood allergen-specific IgE	3 (0-8)
% of children with no positive allergens	37 (30.3)
% of children with $\geq$ 4 positive allergens	54 (43.9)
Median serum C-reactive protein (mg/dL)	0.44 (0.19-1.36)

(continued)

TABLE E1. (Continued)

Features	Value
% of children with serum C-reactive protein $\geq$ 0.25	77 (63.1)
Median expired NO (ppb)	17 (10-48)
Potential respiratory pathogens in BAL	
% Any potential pathogen found	39 (31)
% Viral pathogens	29 (23.6)
% Bacterial pathogen	18 (14.5)
% Concurrent viral and bacterial pathogens	8 (6.5)
% <i>Enteroviridae</i> detected	19 (15.4)
% Non- <i>Enteroviridae</i> detected	10 (8.1)
% <i>Streptococcus pneumoniae</i> cultured	6 (4.8)
% <i>Hemophilus influenzae</i> cultured	4 (3.2)
% <i>Moraxella catarrhalis</i> cultured	4 (3.2)

ACT, Asthma Control Test; BMI, body mass index; cACT, Childhood Asthma Control Test; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of FVC; ICU, intensive care unit.

TABLE E2. Prevalence of comorbid diagnoses by BAL granulocyte pattern in 126 children with poorly controlled, treatment-refractory asthma

Comorbid Diagnoses	Isolated eosinophilia (n = 12)	Isolated neutrophilia (n = 20)	Mixed granulocytic (n = 28)	Pauci-granulocytic (n = 66)
Obesity (n = 18; 14%)*	1 (8)†	2 (10)	3 (11)	12 (18)
Mild prematurity (EGA 36-38 wk) (n = 21; 17%)	3 (25)	3 (15)	5 (18)	10 (16)
Sleep-disordered breathing (n = 12; 9%)	0	2 (10)	4 (14)	6 (9)
Vocal cord dysfunction (n = 7; 6%)	1 (10)	1 (5)	1 (4)	4 (6)
Chronic sinusitis (n = 11; 9%)	1 (8)	0	5 (18)	5 (8)
GERD (n = 31; 25%)	2 (17)	4 (20)	8 (29)	17 (26)
Intermittent aspiration (n = 8; 6%)	1 (8)	3 (15)	1 (4)	3 (5)
Eosinophilic esophagitis (n = 7; 6%)	1 (8)	0	3 (11)	3 (5)
Type I laryngeal clefts (n = 17; 13%)	3 (25)	6 (30)‡	2 (7)	6 (9)
Trachea-broncho-malacia (n = 7; 6%)	0	1 (5)	3 (11)	3 (5)
Right middle lobe syndrome (n = 2; 2%)	0	0	1 (4)	1 (2)
Absent ciliary motion (n = 3; 2%)	0	2 (10)	1 (4)	0

EGA, Estimated gestational age; GERD, gastroesophageal reflux disease.

*Row percent.

†Column percent.

‡ $P = .04$; Pearson χ^2 .

TABLE E3. Features of children with problematic asthma compared according to detection of potential pathogens in BAL

Feature	Any potential pathogen detected	Potential pathogens not detected	P
Sample, n (%)	39 (31)	87 (69)	NA
Granulocyte patterns, n (%)			.0001
Isolated eosinophilia	1 (2.6)	11 (12.6)	
Isolated neutrophilia	13 (33.3)	7 (8.0)	
Mixed granulocytic	15 (38.5)	13 (14.9)	
Pauci-granulocytic	10 (25.6)	56 (64.4)	
Selected BAL constituents			
Median total cell count ($\times 10^6$)	1.9 (1.08-4.06)	1.20 (0.56-2.20)	.005
Macrophages (%)	60.0 (24.0-76.0)	73.0 (53.0-87.0)	.01
Neutrophils (%)	12.0 (4.0-42.0)	2.0 (1.0-5.0)	.0001
Eosinophils (%)	0 (0-3.0)	0 (0-1.0)	NS
Lymphocytes (%)	4.0 (1.0-7.0)	2.0 (1.0-4.0)	.05
Ciliated epithelial cells (%)	10 (3.0-19.0)	15 (7.0-29.0)	.02
Median age (y)	8.2 (6.6-11.1)	11.5 (8.5-14.7)	.0001
Sex: male, n (%)	23 (59.0)	53 (60.1)	NS
Race, n (%)			NS
White	26 (66.7)	46 (52.9)	
Black	11 (28.2)	34 (39.1)	
Mixed	2 (5.1)	7 (8.0)	
Mean ACT/cACT scores	14.1 $\pm$ 6.8	16.0 $\pm$ 5.1	NS
Hospital admission in past year, n (%)	21 (55.3)	68 (78.2)	.009
Median duration of asthma (mo)	77 (66.0-105.7)	111 (68.0-159.0)	.01
Median no. of daily controllers	2.0 (1.0-3.0)	3.0 (2.0-4.0)	NS
Median daily ICS dose (μ g fluticasone)	720 (205-800)	800 (320-800)	NS
Maintenance prednisone, n (%)	8 (20.5)	26 (29.9)	NS
Mean pre-BD FEV ₁ %	93.3 $\pm$ 22.0	85.5 $\pm$ 21.2	NS
Mean post-BD FEV ₁ %	96.5 $\pm$ 18.7	94.1 $\pm$ 24.7	NS
Mean % change FEV ₁ post-BD	9.6 $\pm$ 11.1	15.7 $\pm$ 16.8	NS
Mean pre-BD FVC %	99.0 $\pm$ 17.2	97.0 $\pm$ 19.8	NS
Mean post-BD FVC %	100.9 $\pm$ 15.1	104.1 $\pm$ 19.8	NS
Mean pre-BD FEV ₁ /FVC %	93.2 $\pm$ 13.9	87.2 $\pm$ 11.6	.02
Mean post-BD FEV ₁ /FVC %	94.9 $\pm$ 10.2	91.7 $\pm$ 9.9	NS
Mean pre-BD FEF ₂₅₋₇₅ %	83.2 $\pm$ 40.9	67.3 $\pm$ 30.4	.03
Mean post-BD FEF ₂₅₋₇₅ %	91.0 $\pm$ 36.4	81.6 $\pm$ 31.9	NS
Pre-BD airflow limitation, n (%)*	12 (40.0)	42 (55.3)	NS
Post-BD airflow limitation, n (%)	7 (33.3)	18 (36.0)	NS
Median % blood eosinophils	4.0 (1.0-7.5)	4.0 (2.0-8.0)	NS
Median absolute blood eosinophil count (no. of cells/ μ L)	290 (95-575)	260 (110-555)	NS
Absolute blood eosinophils > 300 cells/ μ L, n (%)	19 (50.0)	39 (45.3)	NS
Median absolute blood neutrophil count (no. of cells/ μ L)	3500 (2720-5210)	3010 (2060-4380)	NS
Geometric mean total IgE (IU/mL)	95.9	255.9	.006
Median no. of positive blood allergen-specific IgE tests	0 (0-3.7)	4 (1.0-10.2)	.0001
Children with no positive blood allergens, n (%)	19 (52.8)	18 (20.9)	.0001
Children with ≥ 4 positive blood allergens, n (%)	9 (24.3)	45 (52.3)	.004
Median serum C-reactive protein	0.65 (0.24-1.82)	0.40 (0.18-1.33)	NS
Children with C-reactive protein ≥ 0.25 , n (%)	27 (73.0)	50 (58.8)	NS
Median expired NO (ppb)	9 (5.2-15.7)	33 (11.0-51.0)	.06
Laryngeal clefts, n (%)	6 (15.4)	11 (12.6)	NS
Airway malacia, n (%)	1 (2.6)	6 (5.9)	NS
Absent ciliary motion	3 (9.4)	1 (1.3)	.03

ACT, Asthma Control Test; cACT, Childhood Asthma Control Test; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of FVC; NA, not applicable/available; NS, not significant.*FEV₁/FVC < 90% predicted.

TABLE E4. Features of children with isolated BAL neutrophilia compared according to detection of potential pathogens

Feature	Any potential pathogen detected	Potential pathogens not detected	P
Sample, n (%)	13 (65.0)	7 (35.0)	NA
Selected BAL constituents	1.90 (1.25-12.0)	1.68 (1.20-3.87)	NS
Median total cell count ($\times 10^6$)	68.0 (32.0-81.5)	54.0 (42.0-83.0)	NS
Macrophages (%)	13.0 (7.0-42.0)	7.0 (7.0-25.0)	NS
Neutrophils (%)	0	0	NS
Eosinophils (%)	4.0 (1.0-5.5)	3.0 (0-6.0)	NS
Lymphocytes (%)	10 (2.0-12.0)	11 (4.0-21.0)	
Ciliated epithelial cells (%)			
Median age (y)	7.0 (6.1-8.4)	11.2 (8.1-13.3)	.03
Sex: male, n (%) column percent	7 (53.8)	5 (71.4)	NS
Race, n (%)			NS
White	11 (84.6)	5 (71.4)	
Black	2 (15.4)	2 (28.6)	
Mixed	0	0	
Mean ACT/cACT scores	14.7 $\pm$ 7.7	17.5 $\pm$ 9.0	NS
Hospital admission in past year, n (%)	5 (41.7)	5 (71.4)	NS
Median duration of asthma (mo)	71 (61.0-78.0)	102 (80.0-149.2)	0.04
Median no. of daily controllers	2.0 (1.0-2.5)	2.0 (2.0-3.0)	NS
Median daily ICS dose (μ g fluticasone)	400 (160-800)	320 (320-800)	NS
Maintenance prednisone, n (%)	1 (7.7)	1 (14.3)	NS
Mean pre-BD FEV ₁ %	98.5 $\pm$ 24.5	101.0 $\pm$ 8.8	NS
Mean post-BD FEV ₁ %	99.7 $\pm$ 21.4	113.0 $\pm$ 7.1	NS
Mean % change FEV ₁ post-BD	4.8 $\pm$ 10.8	14.5 $\pm$ 2.1	NS
Mean pre-BD FVC %	99.4 $\pm$ 22.3	107.0 $\pm$ 12.4	NS
Mean post-BD FVC %	101.8 $\pm$ 22.6	120.0 $\pm$ 18.3	NS
Mean pre-BD FEV ₁ /FVC %	97.5 $\pm$ 12.1	91.2 $\pm$ 12.0	NS
Mean post-BD FEV ₁ /FVC %	297.3 $\pm$ 7.3	93.0 $\pm$ 18.3	NS
Mean pre-BD FEF ₂₅₋₇₅ %	101.0 $\pm$ 39.2	84.0 $\pm$ 25.4	NS
Mean post-BD FEF ₂₅₋₇₅ %	98.4 $\pm$ 28.9	108.5 $\pm$ 47.3	NS
Pre-BD airflow limitation, n (%)*	2 (22.2)	2 (40.0)	NS
Post-BD airflow limitation, n (%)	1 (14.3)	1 (50.0)	NS
Median % blood eosinophils	2.0 (1.0-5.7)	3.0 (1.0-4.0)	NS
Median absolute blood eosinophil count (no. of cells/ μ L)	165 (75-520)	170 (90-240)	NS
Absolute blood eosinophils > 300 cells/ μ L, n (%)	4 (33.3)	1 (14.3)	NS
Median absolute blood neutrophil count (no. of cells/ μ L)	3680 (2732-7470)	4030 (2960-7900)	NS
Geometric mean total IgE (IU/mL)	42	93	NS
Median no. of positive blood allergen-specific IgE tests	0 (0-3)	3 (1-3)	NS
Children with no positive blood allergens, n (%)	6 (54.5)	1 (14.3)	.08
Children with $\geq$ 4 positive blood allergens, n (%)	2 (16.7)	1 (14.3)	NS
Median serum C-reactive protein (mg/dL)	0.69 (0.22-1.49)	1.03 (0.19-1.64)	NS
Children with C-reactive protein $\geq$ 0.25, n (%)	9 (75.0)	5 (71.4)	NS
Laryngeal clefts, n (%)	2 (15.4)	4 (57.1)	.05
Airway malacia, n (%)	0	1 (14.3)	NS
Absent ciliary motion	2 (18.2)	1 (16.7)	NS

ACT, Asthma Control Test; cACT, Childhood Asthma Control Test; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of FVC; NA, not applicable/available; NS, not significant.
*FEV₁/FVC < 90% predicted.

TABLE E5. Subanalysis of selected phenotypic features of 33 children with prednisone treatment excluded/total sample

Features	Isolated eosinophilia 7.4/9.5%	Isolated neutrophilia 18.5/15.9%	Mixed granulocytic 16.7/22.2%	Pauci-granulocytic 57.4/52.4%
BAL Total cell count ($\times 10^6$ cells)	2.4/1.78	2.07*/1.79	1.55/1.61	1.18/1.08
BAL eosinophil %	2/4	0/0	2/3	0/0
BAL neutrophil %	1/1	14/12	19/23	1/1
Pre-BD FEV ₁ %	80/70	99/98	91/79	93/91
Absolute blood eosinophils (cells/ μ L)	515†/395	170/170	430†/480	185/180
Geometric mean total blood IgE (IU/mL)	199‡/297	39/55	161/322	127/194
Any microbe detected %	12/8	60§/65	56/54	16/15
Any virus detected %	0/0	40 /45	41/46	10/11
Any bacteria detected %	12/8	35¶/35	23/18	10/8

* $P = .01$ isolated neutrophilia vs pauci-granulocytic.† $P = .005$ vs isolated neutrophilia and vs pauci-granulocytic.‡ $P = .05$ vs isolated neutrophilia.§ $P = .001$; standardized residual = 2.4.|| $P = .001$; standardized residual = 2.1.¶ $P = .05$; standardized residual = 2.0.**TABLE E6.** Subanalysis of selected phenotypic features of 88 children treated with ICS/LABA therapy

Features	Isolated eosinophilia (n = 9; 10.2%)	Isolated neutrophilia (n = 13; 14.9%)	Mixed granulocytic (n = 19; 21.6%)	Pauci-granulocytic (n = 47; 53.4%)
Median age (y)	11.0 $\pm$ 2.7	8.5 $\pm$ 5.8	9.8 $\pm$ 7.5	12.3 $\pm$ 6.6
Nonwhite race, n (%)	7 (77.8)*	3 (23.1)	10 (52.6)	23 (48.9)
Hospitalized in past year, n (%)	9 (100)†	7 (53.8)	15 (78.9)	37 (78.7)
No. of daily controller medications	4 $\pm$ 2	2 $\pm$ 1‡	4 $\pm$ 1	3 $\pm$ 1
Maintenance prednisone treatment, n (%)	5 (55.6)	2 (15.4)	11 (57.9)§	14 (29.8)
BAL cell count ($\times 10^6$)	1.75 $\pm$ 2.12	2.25 $\pm$ 6.75	2.21 $\pm$ 4.42	1.20 $\pm$ 1.64
Pre-BD FEV ₁ %	67 $\pm$ 15¶	101 $\pm$ 18	76 $\pm$ 24#	86 $\pm$ 17
Post-BD FEV ₁ % change	24 $\pm$ 14	4 $\pm$ 11	21 $\pm$ 24	13 $\pm$ 12
Pre-BD FVC %	81 $\pm$ 15**	104 $\pm$ 19	92 $\pm$ 22	98 $\pm$ 16
Pre-BD FEV ₁ /FVC %	82 $\pm$ 9**	96 $\pm$ 13	81 $\pm$ 13††	88 $\pm$ 10
Absolute blood eosinophils (cells/ μ L)	420 $\pm$ 520	165 $\pm$ 200	410 $\pm$ 660§	180 $\pm$ 300
Geometric mean total blood IgE (IU/mL)	279 (12-2570)	61 (5-629)	228 (6-4378)	245 (7-4804)
Proportion $\geq$ 4 allergens, n (%)	6 (66.7)	3 (25.0)	12 (63.2)	25 (53.2)
Any BAL pathogen, n (%)	0	8 (61.5)	12 (63.2)‡‡	5 (10.6)
Any BAL virus, n (%)	0	6 (46.2)	10 (52.6)‡‡	3 (6.4)
Any BAL bacteria, n (%)	0	4 (30.8)†	4 (21.1)	3 (6.4)

LABA, Long-acting β -agonist.* $P = .06$.† $P = .04$.‡ $P = .01$, vs mixed granulocytic.§ $P = .03$.|| $P = .03$ vs isolated neutrophilia.¶ $P < .05$ vs isolated neutrophilia and pauci-granulocytic.# $P = .004$ vs isolated neutrophilia.** $P = .02$ vs isolated neutrophilia.†† $P = .007$ vs isolated neutrophilia.‡‡ $P = .0001$.

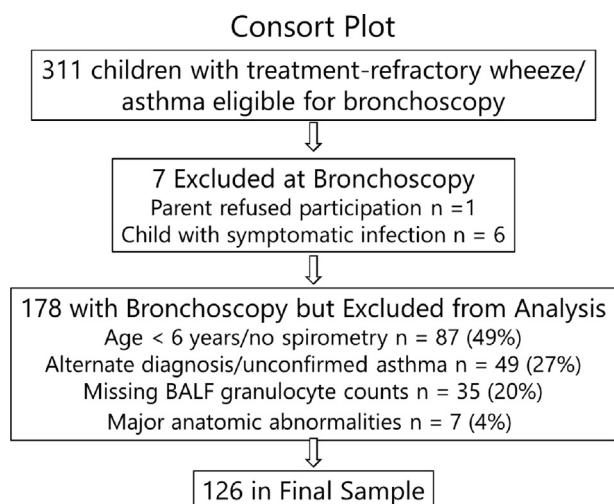


FIGURE E1. Consort plot of children with severe, treatment-refractory, poorly controlled asthma or wheeze eligible for phenotypic characterization procedures and diagnostic bronchoscopy with BAL. *BALF*, BAL fluid.

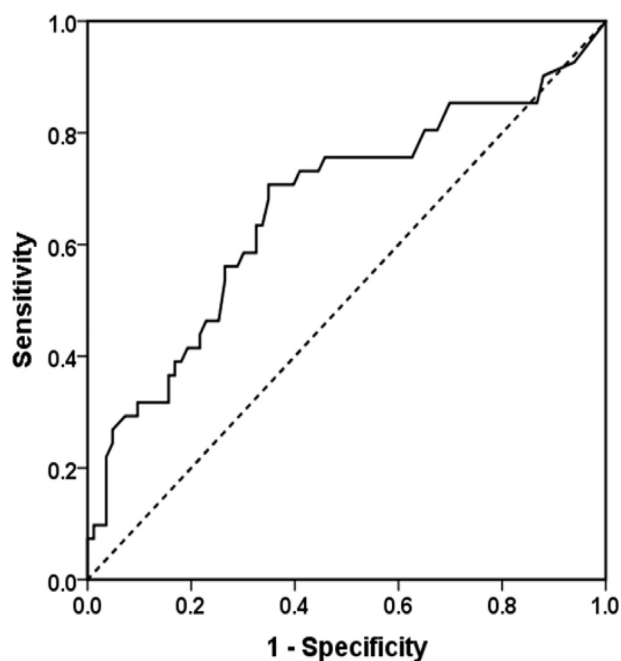


FIGURE E2. ROC curve depicting the specificity and sensitivity of the absolute blood eosinophil count as a predictor of BAL eosinophilia in 124 children with poorly controlled, therapy-resistant asthma. The area under the curve is 0.66, indicating poor performance, although significant with a *P* value of .003.

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