ORIGINAL ARTICLE

Recurrent Severe Preschool Wheeze: From Prespecified Diagnostic Labels to Underlying Endotypes

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Abstract

Rationale: Preschool wheezing is heterogeneous, but the underlying mechanisms are poorly understood.

Objectives: To investigate lower airway inflammation and infection in preschool children with different clinical diagnoses undergoing elective bronchoscopy and BAL.

Methods: We recruited 136 children aged 1–5 years (105 with recurrent severe wheeze [RSW]; 31 with nonwheezing respiratory disease [NWRD]). Children with RSW were assigned as having episodic viral wheeze (EVW) or multiple-trigger wheeze (MTW). We compared lower airway inflammation and infection in different clinical diagnoses and undertook data-driven analyses to determine clusters of pathophysiological features, and we investigated their relationships with prespecified diagnostic labels.

Measurements and Main Results: Blood eosinophil counts and percentages and allergic sensitization were significantly higher in children with RSW than in children with a NWRD. Blood neutrophil counts and percentages, BAL eosinophil and neutrophil percentages, and positive bacterial culture and virus detection rates were similar between groups. However, pathogen distribution differed significantly, with higher detection of rhinovirus in children with RSW and higher detection of *Moraxella* in sensitized children with RSW. Children with EVW and children with MTW did not differ in terms of blood or BAL-sample inflammation, or bacteria or virus detection. The Partition around Medoids algorithm revealed four clusters of pathophysiological features: *1*) atopic (17.9%), *2*) nonatopic with a low infection rate and high use of inhaled corticosteroids (31.3%), *3*) nonatopic with a high infection rate (23.1%), and *4*) nonatopic with a low infection rate and no use of inhaled corticosteroids (27.6%). Cluster allocation differed significantly between the RSW and NWRD groups (RSW was evenly distributed across clusters, and 60% of the NWRD group was assigned to cluster 4; $P < 0.001$). There was no difference in cluster membership between the EVW and MTW groups. Cluster 1 was dominated by *Moraxella* detection ($P = 0.04$), and cluster 3 was dominated by *Haemophilus* or *Staphylococcus* or *Streptococcus* detection ($P = 0.02$).

Conclusions: We identified four clusters of severe preschool wheeze, which were distinguished by using sensitization, peripheral eosinophilia, lower airway neutrophilia, and bacteriology.

Keywords: pediatric wheeze; cluster analysis; infection; inflammation; endotypes

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At a Glance Commentary

Scientific Knowledge on the Subject: The pathophysiological mechanisms of preschool wheeze are poorly understood.

What This Study Adds to the Field: We analyzed the peripheral blood and lower airway infection and inflammation profiles in 136 preschoolers with wheeze or other respiratory diseases and found four distinct clusters. Sensitization, peripheral eosinophilia, and bacteriology findings in induced sputum may be useful biomarkers to stratify treatment.

Wheezing in children under 6 years of age results in significant morbidity, emergency department attendance, and hospitalization (1). Preschool wheezing is heterogeneous and results from multiple causes (2, 3), but the underlying mechanisms are poorly understood (3, 4). The European Respiratory Society Task Force recommends management based on the clinical phenotype, which consists of as-required use of short-acting bronchodilators for episodic viral wheeze (EVW) and regular use of inhaled corticosteroids (ICS) for multiple-trigger wheeze (MTW), although ICS are also considered in patients with frequent/severe EVW (5, 6). However, the distinction between EVW and MTW is sometimes unclear (6, 7), and phenotypes often change over time (7, 8). Despite these limitations, the classification of EVW and MTW has gained widespread acceptance (6).

Some preschool children with wheeze have recurrent severe wheeze (RSW) with multiple exacerbations and hospitalizations (9) and poorer long-term outcomes (10, 11). Most children with RSW are prescribed ICS irrespective of phenotype (12), and discontinuation of treatment is rarely implemented because of disease severity (9).

Evaluation of lower airway inflammation and infection by using bronchoscopy in adult asthma elucidated the pathophysiological mechanisms and facilitated new treatment strategies (13). However, invasive procedures for research purposes are not ethically acceptable in children. The only way to probe the mechanisms is to use data from patients undergoing clinically indicated bronchoscopy. Such assessments have shown inconsistent results, and airway pathology in children with RSW seems to be age dependent (14, 15). For example, we showed no evidence of submucosal airway inflammation and/or remodeling in children aged 3.4–26 months with RSW, even when RSW was atopic (14); however, by the age of 3 years, children with a similar clinical phenotype had pathological features characteristic of asthma in adults and school-aged children, namely submucosal eosinophilia and reticular basement membrane (RBM) thickening (15). Similarly, one study in RSW undertaken at median age of 1 year showed no differences in BAL inflammation (16), and another study at a similar age showed an increased number of total BAL-sample leukocytes in children with wheeze compared with healthy control subjects (17). A study in children aged 2–10 years suggested that bronchodilator-responsive MTW had an airway pathology typical of adult asthma (eosinophilia and RBM thickening) among both children with nonatopic MTW and children with atopic MTW (18). In contrast, analysis of BAL samples from 18 young children with wheeze revealed predominantly neutrophilic airway inflammation (19). This suggests that RSW is multifactorial and is characterized by a complex interplay among airway inflammation, infection, and treatment, but this has not been systematically addressed.

We hypothesized that among preschoolers with severe respiratory disease, the pattern of lower airway inflammation and bacterial and viral infection *1*) differs between those with and without RSW, *2*) differs between those with MTW and those with EVW among those with wheeze, and *3*) is influenced by atopic status and prescribed treatment with ICS. To address our hypotheses, we compared data on lower airway inflammation and infection in preschool children with different clinical diagnoses undergoing elective bronchoscopy. We then undertook an unbiased analysis to determine whether distinct patterns of pathophysiological features could be identified and investigated the relationships between such clusters and prespecified diagnostic labels.

Methods

Detailed methods are presented in the online supplement.

Study Design, Setting, Participants, and Data Sources

We recruited children aged 12–72 months undergoing clinically indicated, elective bronchoscopy for severe respiratory symptoms at the Royal Brompton Hospital (London, United Kingdom). Inclusion and exclusion criteria are shown in Table E1 in the online supplement. Ethical approval was obtained from the National Research Ethics Committee. Written informed consent was obtained from parents or guardians.

Information on symptoms, treatments, demographics, and medical history was collected from the electronic patient records and from validated questionnaires completed by parents or carers.

Peripheral blood was processed for differential leukocyte counts and percentages as well as IgE specific to common inhalant and food allergens. Allergic sensitization was defined as an allergen-specific IgE level $\geqslant$0.35 kUA/L in response to at least one allergen tested.

Definition of Clinical Phenotypes

RSW. Children were assigned at recruitment as having RSW if they had recurrent episodes of physician-confirmed severe wheeze. Within this group, wheeze phenotypes were defined as follows: MTW was defined by symptoms with and apart from acute episodes, and EVW was defined by symptoms only during acute episodes (*see* Table E2 in the online supplement) (5, 6). If the distinction was unclear (details in the online supplement), a wheeze phenotype was not assigned.

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This article has a related editorial.

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Nonwheezing respiratory disease. Children with upper airway symptoms (stridor, barking cough), suspected tracheal compression, and/or recurrent respiratory tract infections but with no wheezing were assigned to the nonwheezing respiratory disease (NWRD) control group (Table E3).

Bronchoscopy and BAL

Fiberoptic bronchoscopy and BAL were performed under general anesthesia (20, 21). A differential cell count was performed on BAL cytospin samples, and macrophages, neutrophils, eosinophils, and lymphomononuclear cells were quantified as the percentage of total leukocytes. Endobronchial biopsies were undertaken when clinically indicated, as previously described (15).

The presence of 21 respiratory pathogens in BAL samples (20 viruses and *Mycoplasma pneumoniae*) was ascertained by using a multiplex real-time PCR assay. The presence of bacteria was determined by using a bacterial culture.

Statistical Analysis

Characteristics of clinical phenotypes. The assessment of differences between clinical phenotypes (RSW vs. NWRD; MTW vs. EVW) was conducted by using *t* tests and ANOVA for normally distributed, continuous data and by conducting Mann-Whitney or Kruskal-Wallis tests with Dunn's correction for multiple comparisons for non–normally distributed data. Categorical data were analyzed by using chi-square or Fisher exact tests. Values are expressed as medians with ranges, and statistical significance was accepted as being indicated by an adjusted P value of <0.05.

Unbiased analysis of pathophysiological features. We used the Partition around Medoids algorithm (22) coupled with Gower's distance for mixed data to classify children into a limited number of homogeneous pathophysiological subtypes on the basis of their similarity in terms of objective biomarkers. We used eight variables across four dimensions (inflammation, infection, sensitization, and therapy with ICS) to derive clusters (Table E4). To account for missing data, we adopted a framework that integrates multiple imputation into cluster analysis (23). The optimal solution was chosen according to the Calinski-Harabasz criterion. The analysis was run in the programming language R (R Foundation for Statistical Computing). The missing data imputation was performed by using the *missMDA* package (24), and cluster analysis was applied by using the package *cluster* (25). We used the *ggplot2* package for data visualization (26).

Results

Participants and Characteristics of the Study Population

We recruited 136 preschool children (105 with RSW and 31 with NWRD); almost all had a BAL sample taken, and ~80% and 95% of these samples were evaluable for cytology and for microbiology and virology, respectively (Table E5).

Table 1 shows characteristics of the study population stratified by clinical phenotypes. There were no differences between those with RSW and those with NWRD in terms of demographic characteristics, environmental exposures, or pre- and perinatal factors, apart from the higher proportion of children with NWRD who were born via cesarean section. The RSW group had a significantly higher frequency of allergic sensitization (30% vs. 4%; $P = 0.008$) and prescription of ICS than the NWRD group.

Children in the RSW group had a lifetime median of six hospitalizations and four oral corticosteroid courses. We assigned 30 of 105 of the patients with RSW as having EVW and assigned 44 of 105 of the patients with RSW as having MTW; 28 children could not be accurately assigned. A similar proportion of children with MTW and children with EVW were sensitized (~30%), and there were no differences in hospitalizations or medication prescriptions. The only significant difference was the parentally reported exposure to passive smoking (41% in the MTW group vs. 10% in the EVW group; $P = 0.01$).

Peripheral Eosinophilia and Lower Airway Inflammation and Infection in the RSW and NWRD Groups

Data on peripheral blood and lower airway inflammation and infection are shown in Table 1 and Figure E1. Blood eosinophil counts and percentages were significantly higher in the RSW group than in the NWRD group (Figure E1A), but there was no difference in other leukocytes (Figures E1B–E1D).

Approximately half of study participants had a positive bacterial culture result and/or BAL-sample viral result, with no difference being shown between the RSW and NWRD groups (Figures E2A and E2B). However, the distribution of pathogens differed, with a higher proportion in the RSW group than in the NWRD group having rhinovirus detected (Figure 1). BAL-sample neutrophil percentages were significantly higher among children with a positive BAL-sample bacterial culture result than in those with a negative BAL-sample bacterial culture result, but this was only applicable in the RSW group (Figure 2A) and did not pertain to the NWRD group (Figure 2B). There were no differences in BAL-sample neutrophil percentages stratified by virus detection (Figures 2C and 2D).

We obtained good-quality airway histological data from biopsies in a limited number of children (37 with RSW and 9 with NWRD). Data on tissue inflammation and remodeling are shown in the online supplement and in Figure E3. Briefly, compared with children with NWRD, children with RSW had significantly increased RBM thickness, and there was a trend toward increased airway smooth muscle mass.

RSW group: sensitization, blood eosinophil counts and percentages, BAL-sample inflammation, bacterial culture, and use of ICS. In the RSW group, blood eosinophil counts and percentages were significantly higher in sensitized children (Figure E4A), but there was no difference between sensitized and nonsensitized children in terms of BAL-sample eosinophil or neutrophil percentages (Figures E4B and E4C). Sensitization status did not affect the frequency of bacterial detection (Figure 3A), but sensitized children with RSW were significantly more likely to have *Moraxella* detected than nonsensitized children (Figure E5). BAL-sample neutrophil percentages were significantly higher in children with a positive BAL-sample bacterial culture result, both among sensitized and nonsensitized children (Figure 3B), with no impact of bacterial infection on BAL-sample eosinophil percentages being demonstrated (Figure 3C).

Use of ICS was reported in almost all sensitized children with RSW and was reported in three-quarters of nonsensitized children with RSW (Figure E6A). Those prescribed ICS had significantly higher BAL-sample eosinophil percentages (Figure E6B); BAL-sample neutrophil percentages did not differ according to use of ICS (Figure E6C). The bacterial culture was positive in 49% of

Table 1. Patient Demographics, Perinatal Factors, Clinical Features, Medications, Environmental Exposures, Inflammation, Infection, and Cluster Membership

	RSW (*N* = *105*)	NWRD (*N* = *31*)	*P*	MTW (*N* = *44*)	EVW (*N* = *30*)	No Phenotype (*N* = *31*)	*P*
Demographics							
Sex, M, *n* (%)	66 (63)	15 (48)	0.21	30 (68)	17 (57)	19 (61.3)	0.59
Age, mo, $\tilde{x}$ (range)	34.8 (13–70)	33.1 (14–64)	0.39	30.5 (13–70)	34.9 (14–69)	38.8 (14–69)	0.41
Weight, kg, $\tilde{x}$ (range [*n*])	14.3 (9–26 [82])	14.0 (8–19 [22])	0.35	14.2 (9–22 [40])	14.3 (11–23 [26])	14.4 (9–26 [16])	0.79
Height, cm, $\tilde{x}$ (range [*n*])	94.3 (56–121 [83])	91.8 (73–113 [23])	0.42	89.8 (56–117 [38])	94.3 (81–115 [27])	97 (83–121 [18])	0.09
Ethnicity, *n*	62/105	11/31	—	34/44	22/30	6/31	—
White, *n* (%)	43 (69)	10 (91)	—	27 (79)	13 (59)	3 (50)	—
Black, *n* (%)	5 (8)	0 (0)	—	3 (9)	2 (9)	0 (0)	—
Asian, *n* (%)	6 (10)	0 (0)	—	1 (3)	4 (18)	1 (17)	—
Mixed/other, *n* (%)	8 (13)	1 (9)	—	3 (9)	3 (14)	2 (33)	—
Perinatal factors							
Gestation, wk, $\tilde{x}$ (range [*n*]) or mean ± SD (range [*n*])	39 (36–42 [93])	40 (36–41 [23])	0.68	39 ± 1 (36–42 [44])	39 ± 6 (36–42 [29])	39 (36–42 [20])	0.26
Vaginal delivery, *n* (%)	51/77 (66)	6/17 (35)	0.03	29/37 (78)	17/27 (63)	5/13 (38)	0.03
Birth weight, kg, $\tilde{x}$ (range [*n*])	3.2 (1.4–4.3 [82])	3.4(1.8–4.1 [16])	0.74	3.2 (2.2–4.3 [40])	3.5(1.4–4.2 [27])	2.9 (1.9–3.6 [14])	0.004
Oxygen required, *n* (%)	3/75 (4)	1/13 (8)	0.48	2/42 (5)	0/26 (0)	1/7 (14)	0.21
Ventilation, *n* (%)	2/76 (3)	2/14 (14)	0.11	1/43 (2)	0/26 (0)	1/7 (14)	0.11
Breastfed (ever), *n* (%)	49/76 (64)	9/13 (69)	0.99	24/35 (69)	17/26 (65)	8/15 (53)	0.58
Breastfeeding duration, mo, $\tilde{x}$ (range [*n*])	4 (0–24 [48])	4 (0–18 [9])	0.38	3 (0–12 [25])	6 (0–24 [18])	3 (1–9 [5])	0.25
Clinical features							
Allergic sensitization, *n* (%)	30/100 (30)	1/23 (4)	0.008	12/42 (29)	9/28 (32)	9/30 (30)	0.95
TRACK score, $\tilde{x}$ (range [*n*])	17 (4–26 [15])	N/A	N/A	16 (4–23 [9])	23 (5–26 [5])	0 (0–0 [0])	0.09
PACQLQ score, $\tilde{x}$ (range [*n*])	4.1 (1–7 [47])	N/A	N/A	3.8 (1–7 [31])	5.3 (1–7 [16])	0 (0–0 [0])	0.55
Hospitalizations for wheeze, $\tilde{x}$ (range [*n*])	6 (0–100 [59])	N/A	N/A	6 (0–100 [31])	6 (0–21 [22])	4 (2–6 [6])	0.27
Abnormal pH result, *n* (%)	22/80 (27.5)	5/26 (19.2)	0.45	5/33 (15.2)	7/25 (28)	10/22 (45)	0.047
Medications							
Inhaled corticosteroids, *n* (%)	78/104 (75)	5/29 (17)	0.001	32/43 (75)	21/30 (70)	20/22 (91)	0.18
OCS ever *n* (%)	68/78 (87)	11/15 (73)	0.23	35/39 (90)	18/23 (78)	15/16 (94)	0.29
OCS courses, $\tilde{x}$ (range [*n*])	4 (0–150 [61])	1 (0–5 [11])	0.007	5 (0–150 [34])	5 (0–20 [22])	3 (1–6 [5])	0.64
Salbutamol (ever), *n* (%)	101/103 (98)	15/18 (83)	0.02	43/44 (98)	29/30 (97)	29/29 (100)	0.64
Montelukast (ever), *n* (%)	81/89 (91)	10/16 (63)	0.007	36/41 (88)	26/29 (90)	19/19 (100)	0.29
Oral antibiotics (ever), *n* (%)	75/76 (99)	13/16 (81)	0.02	33/34 (97)	22/22 (100)	21/21 (100)	0.53
Antibiotic courses, $\tilde{x}$ (range [*n*])	2 (0–21 [76])	2 (0–30 [16])	0.51	3 (0–21 [33])	2 (1–13 [22])	2 (1–9 [21])	0.27
Environmental exposures							
Passive smoking, *n* (%)	28/98 (30)	5/18 (28)	0.99	17/42 (41)	3/29 (10)	8/22 (36)	0.02
Urban environment, *n* (%)	47/60 (78)	11/13 (85)	0.99	27/35 (77)	20/24 (83)	0/1 (0)	0.14
Pets, *n* (%)	26/86 (30)	9/18 (50)	0.17	16/40 (40)	8/29 (28)	2/17 (12)	0.10
Siblings, $\tilde{x}$ (range [*n*])	1 (0–5 [89])	1 (0–10 [24])	0.82	1 (0–5 [40])	1 (0–4 [28])	2 (0–5 [21])	0.26
Peripheral blood							
Eosinophil count, $\times 10^9$/L, $\tilde{x}$ (range [*n*])	0.4 (0–2 [99])	0.2 (0–1.2 [28])	0.009	0.4 (0–2 [43])	0.45 (0.1–2 [26])	0.45 (0.1–1.8 [30])	0.71
Eosinophil %, $\tilde{x}$ (range [n])	4.1 (0–16 [99])	2.0 (1–10 [28])	0.004	4.2 (0–15 [43])	4.3 (1–14 [26])	3.9 (1–14 [30])	0.85
Neutrophil count, $\times 10^9$/L, $\tilde{x}$ (range [n])	4.5 (1–13 [99])	4.5 (3–11 [28])	0.97	4.8 (1–11 [43])	4.2 (2–13 [26])	4.6 (2–8 [30])	0.67
Neutrophil %, $\tilde{x}$ (range [*n*])	41.7 (14–81 [99])	42.6 (23–76 [28])	0.71	41.7 (14–81 [43])	38.8 (16–67 [26])	44.3 (23–62 [30])	0.54
Lymphocyte %, $\tilde{x}$ (range [*n*])	46.3 (11–74 [96])	47.4 (19–84 [25])	0.7	46.7 (11–70 [43])	47.4 (22–74 [25])	42.4 (25–61 [28])	0.52
Monocyte %, $\tilde{x}$ (range [*n*])	7.0 (2–17 [96])	7.2 (5–17 [25])	0.61	7.0 (2–17 [43])	7.0 (5–14 [25])	7.0 (4–15 [28])	0.80
IgE, IU/ml, $\tilde{x}$ (range [*n*])	21 (0–1575 [93])	10 (1–1,000 [30])	0.09	20 (0–1,575 [40])	14 (1–990 [27])	30 (1–826 [26])	0.84
BAL samples							
Eosinophil %, $\tilde{x}$ (range [*n*])	1.3 (0–16 [82])	0.5 (0–8 [28])	0.09	1.3 (0–16 [37])	0.9 (0–9 [25])	2 (0–6 [20])	0.22
Neutrophil %, $\tilde{x}$ (range [*n*])	7.5 (0–82 [82])	16.9 (1–82 [28])	0.09	12.3 (0–63 [37])	6.3 (1–82 [25])	6 (1–68 [20])	0.18
Lymphocyte %, $\tilde{x}$ (range [*n*])	11.2 (2–59 [82])	9.1 (0.7–38 [28])	0.44	13.3 (3–59 [37])	9 (3–33 [25])	11 (2–26 [20])	0.14
Macrophage %, $\tilde{x}$ (range [*n*])	71.7 (6–94 [82])	65.4 (10–93 [28])	0.35	62.1 (18–92 [37])	77.7 (6–93 [25])	72.3 (18–94 [20])	0.04
Positive bacteriology result, *n* (%)	45/102 (44)	14/31 (45)	0.99	22/43 (51)	13/27 (45)	10/30 (33)	0.32
Positive viral PCR result, *n* (%)	44/99 (44)	16/29 (55)	0.4	21/42 (50)	8/27 (30)	15/30 (50)	0.19

Definition of abbreviations: EVW = episodic viral wheeze; MTW = multiple-trigger wheeze; N/A = not applicable; NWRD = nonwheezing respiratory disease; OCS = oral corticosteroids; PACQLQ = Pediatric Asthma Caregiver's Quality of Life Questionnaire; RSW = recurrent severe wheeze; TRACK = Test for Respiratory and Asthma Control in Kids; $\tilde{x}$ = median.

For further information on the TRACK and PACQLQ, *see* References 23 and 24, respectively. Hospitalizations were for wheeze only. For categorical data, statistics represent the result of chi-square or two-tailed Fisher exact tests if any group *n* was <5 for contingency tables. For continuous data, statistics represent the results of D'Agostino and Pearson normality tests followed by the Student's *t* tests or ANOVA for parametric data or Mann-Whitney or Kruskal-Wallis tests for nonparametric data. A *P* value of <0.05 was used to define statistical significance.

children prescribed ICS and in 32% of those not prescribed ICS ($P = 0.14$; Figure 3D). Among those prescribed ICS, BAL-sample neutrophil percentages were significantly higher in children with a positive BAL-sample bacterial culture result (Figure 3E), with no difference in BAL-sample eosinophil percentages being shown (Figure 3F).

There was no difference in the blood or BAL-sample eosinophil or neutrophil counts and percentages or percentages between the EVW and MTW groups (Figures E7A and E7B); the EVW group had significantly more BAL-sample macrophages (77 vs. 62.1; $P = 0.03$; Figure E7C).

Pathophysiological Clusters of Severe Preschool Airway Diseases

We included 134 of 136 participants in the unbiased analysis. Detailed information on missing data (Figure E8), imputation, application of the Partition around Medoids algorithm, and the choice of the optimal solution is presented in the online supplement. We selected four as the optimal number of clusters (Figure E9). There was a high degree of certainty in the classification, and most individuals had a probability of their cluster membership close to 1 (Figure E10).

On the basis of the distributions of the eight variables within each cluster (Figure 4), we qualitatively labeled the clusters as follows: *1*) atopic ($n = 24$ of 134, 17.9%), *2*) nonatopic with a low infection rate and high use of ICS ($n = 42$ of 134, 31.3%, *3*) nonatopic with a high infection rate ($n = 31$ of 134, 23.1%), and *4*) nonatopic with a low infection rate and no use of ICS ($n = 37$ of 134, 27.6%). Cluster 1 was characterized by the highest prevalence of sensitization (100.0%) and the highest blood eosinophil counts and percentages (mean, 5.54%; SD, 2.86%), high use of ICS (91.7%), and a moderate rate of bacterial (69.5%) and viral detection (56.5%). Children in cluster 2 had low BAL-sample neutrophils (mean, 9.44%; SD, 13.89%), a low rate of positive bacteriology results (17.1%), and the lowest viral detection rate (15.0%). All children in this cluster were prescribed ICS. Cluster 3 was characterized by the highest rate of bacterial and viral infection (96.8% and 86.7%, respectively) and the highest BAL-sample neutrophils (mean, 31.7%; SD, 25.11%); 67.7% of children in this cluster used ICS. A striking characteristic of cluster 4 was that not a single child was using ICS; most children in this cluster were nonatopic.

We repeated the analysis using only children within the RSW group. Results confirmed the same four profiles (Figure E11).

Demographic characteristics, clinical characteristics, infection rates, and features of the four clusters are shown in Table 2. The most discriminant variables in the classification were blood eosinophil counts and percentages, BAL-sample neutrophil percentages, sensitization, use of ICS, positive bacteriology results, and positive viral PCR results, for which significant differences were observed among clusters (Table 2; Figure 4). Children in cluster 1 were older and had a higher number of hospitalizations. Significant associations were found between cluster membership and symptoms, with all children in cluster 1 reporting wheezing; in contrast, persistent cough was common in cluster 4.

Mosaic plots of the distribution of bacteria and viruses in different clusters are shown in Figure E12. The distribution of bacteria differed significantly, with infection in cluster 1 being dominated by *Moraxella* ($P = 0.04$) and infection in cluster 3 being

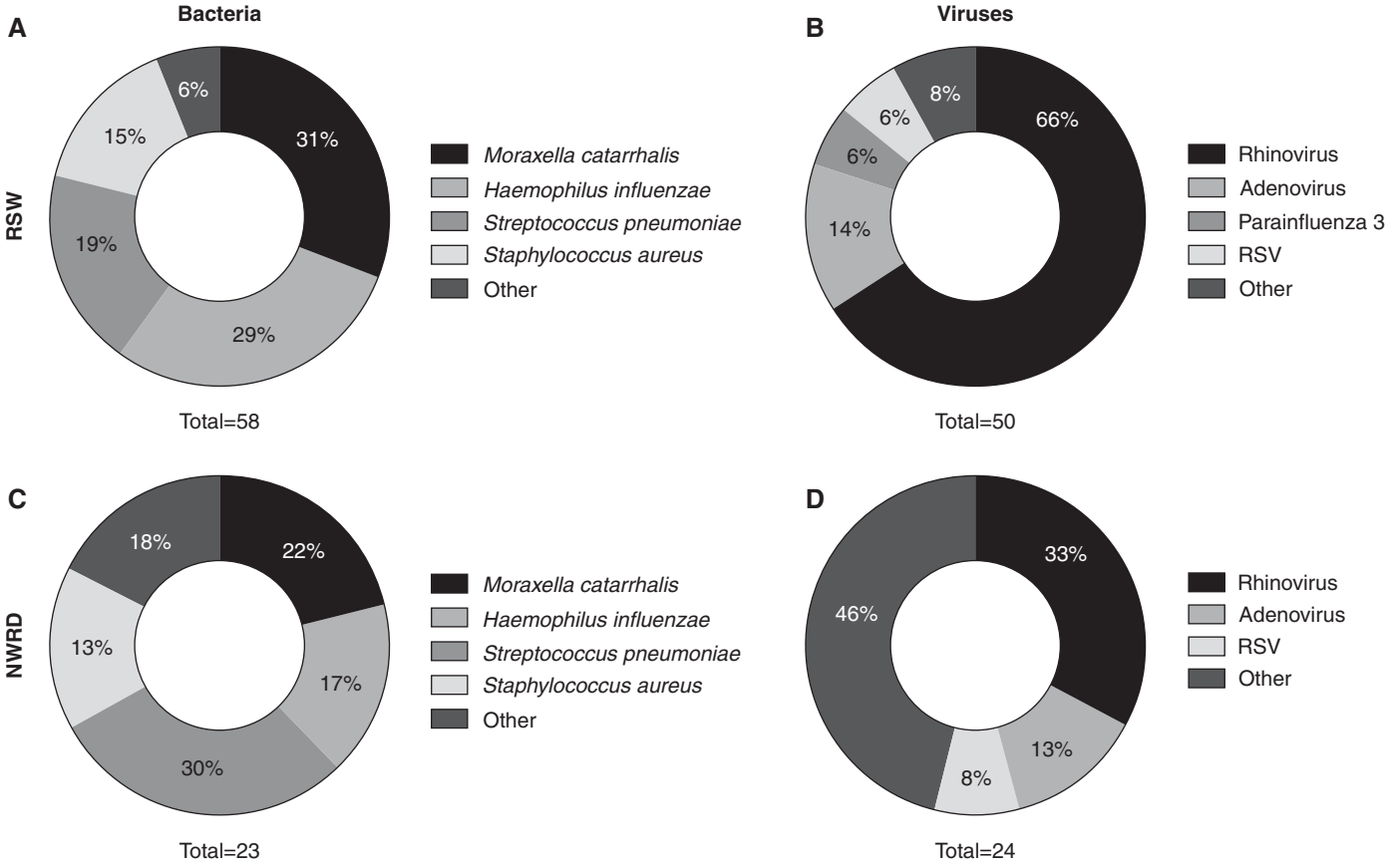

Figure 1. Distribution of BAL-sample pathogens in recurrent severe wheeze (RSW) and nonwheezing respiratory disease (NWRD). Distribution of pathogens in BAL samples from children with RSW and positive results from (*A*) bacterial cultures (45 children) and (*B*) viral PCR analyses (44 children). Distribution of pathogens in BAL samples from children with NWRD and positive results from (*C*) bacterial cultures (14 children) and (*D*) viral PCR analyses (16 children). Multiple children had positive results for more than one pathogen in their BAL samples; hence, the total number of species is given under each graph. This differs from the total number of children with a positive infection rate. Not all participants had samples; results are only shown for samples successfully processed for infection results. RSV = respiratory syncytial virus.

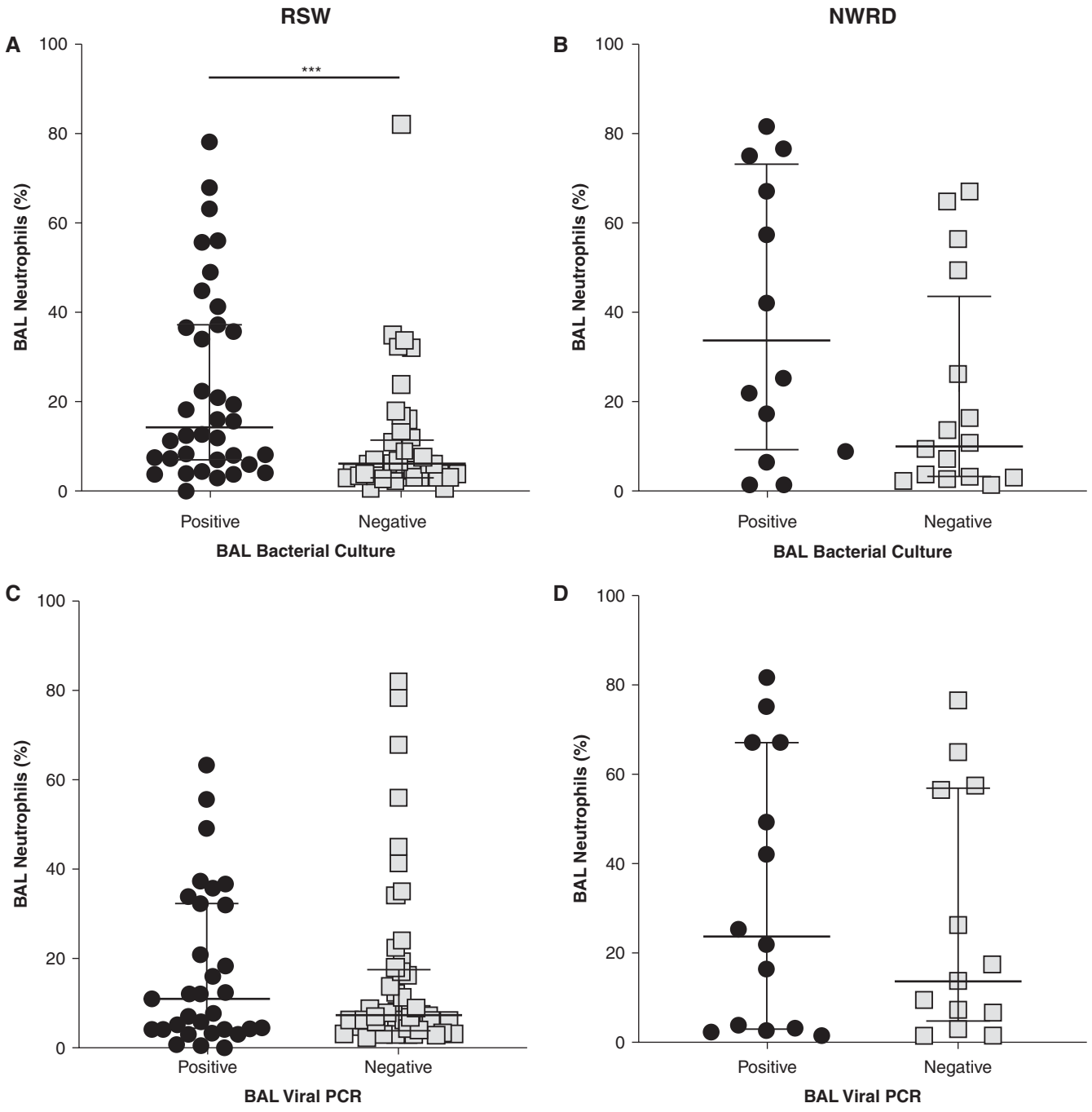

Figure 2. BAL-sample neutrophilic inflammation and viral or bacterial detection in recurrent severe wheeze (RSW) and nonwheezing respiratory disease (NWRD). Percentage of BAL-sample neutrophils according to BAL-sample bacterial culture in (*A*) RSW (positive $n = 36$, negative $n = 45$) and (*B*) NWRD (positive $n = 12$, negative $n = 16$). Percentage of BAL-sample neutrophils according to BAL-sample viral PCR in (*C*) RSW (positive $n = 31$, negative $n = 49$) and (*D*) NWRD (positive $n = 14$, negative $n = 13$). Black circles represent children with positive BAL-sample infection results, and gray squares represent children with negative BAL-sample infection results. Not all participants had samples, as these were only taken if clinically indicated; results are only shown for samples successfully processed for both infection results and cellular inflammation. The *n* for each group is stated. Statistics represent (*A* and *C*) the results of D'Agostino and Pearson normality tests followed by Mann-Whitney tests for nonparametric data or (*B* and *D*) the results of Student's *t* tests for parametric data. ***$P < 0.001$.

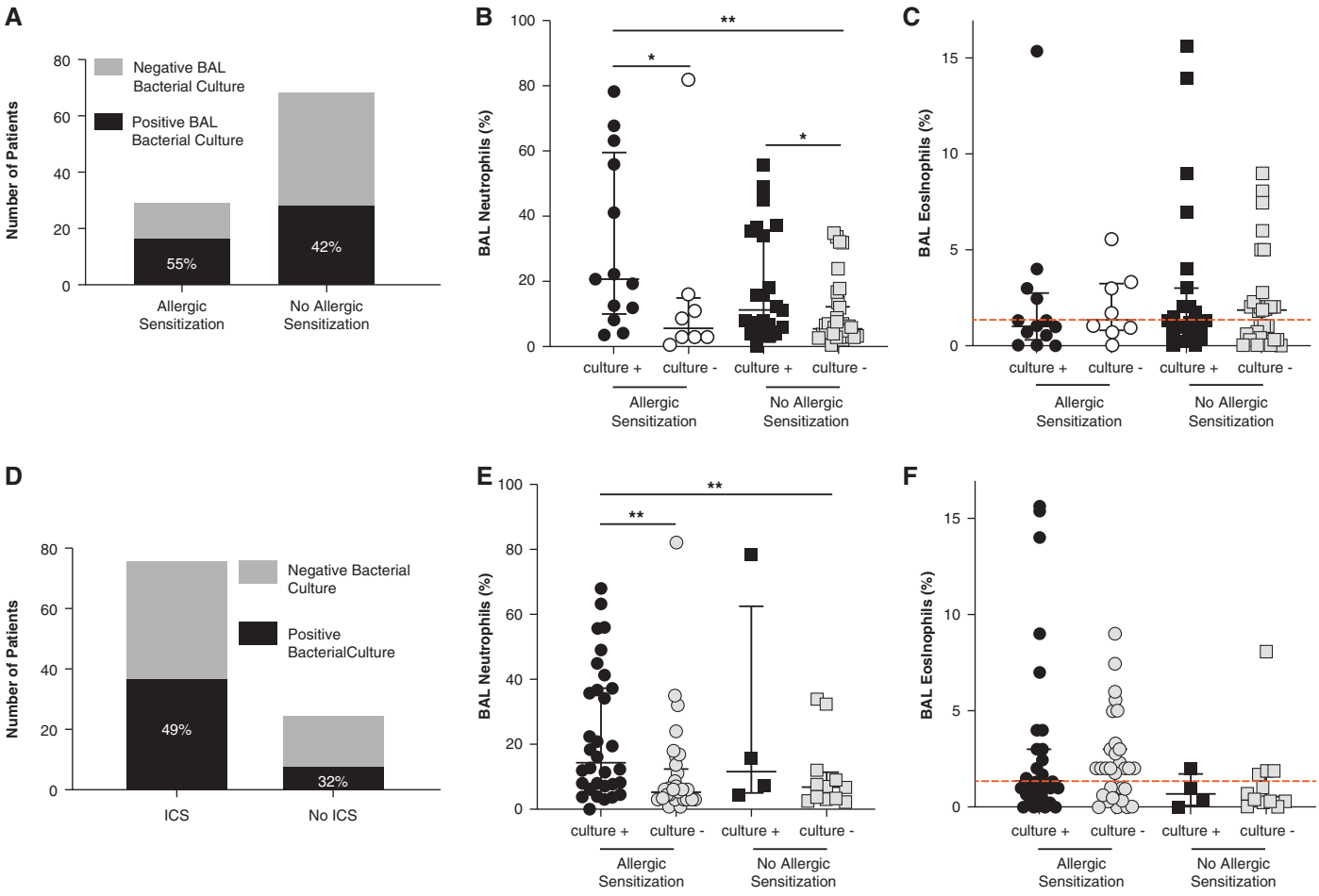

Figure 3. Relationship among allergic sensitization, use of inhaled corticosteroids (ICS), BAL-sample inflammation, and bacterial culture results in recurrent severe wheeze (RSW). (*A*) Bacterial culture results for RSW according to allergic sensitization. Black bars represent the proportion of patients with positive bacterial culture results ($n = 16$ with allergic sensitization, $n = 28$ with no allergic sensitization), and gray bars represent the proportion of patients with negative bacterial culture results ($n = 13$ with allergic sensitization, $n = 40$ with no allergic sensitization). (*B* and *C*) RSW BAL-sample inflammation according to BAL-sample bacterial culture results and allergic sensitization for (*B*) BAL-sample neutrophil percentages and (*C*) BAL-sample eosinophil percentages. Black circles represent children with RSW with positive bacterial culture results and allergic sensitization ($n = 13$), gray circles represent children with RSW with negative bacterial culture results and allergic sensitization ($n = 8$), black squares represent children with RSW with positive bacterial culture results and no allergic sensitization ($n = 23$), and gray squares represent children with RSW with negative bacterial culture results and no allergic sensitization ($n = 24$). (*D*) Bacterial culture results for RSW according to prescription of ICS. Black bars represent the proportion of patients with a positive bacterial culture result ($n = 37$ prescribed ICS, $n = 8$ not prescribed ICS), and gray bars represent the proportion of patients with a negative bacterial culture result ($n = 39$ prescribed ICS, $n = 17$ not prescribed ICS). (*E* and *F*) RSW BAL-sample inflammation according to BAL-sample bacterial culture results and prescription of ICS for (*E*) BAL-sample neutrophil percentages and (*F*) BAL-sample eosinophil percentages. Black circles represent children with RSW with positive bacterial culture results who were prescribed ICS ($n = 32$), gray circles represent children with RSW with negative bacterial culture results who prescribed ICS ($n = 33$), black squares represent children with RSW with positive bacterial culture results who were not prescribed ICS ($n = 4$), and gray squares represent children with RSW with negative bacterial culture results who were not prescribed ICS ($n = 12$). Not all participants had BAL samples, as these were only taken if clinically indicated; results are shown for samples successfully processed for cellular inflammation and/or bacterial infection in patients whose prescription of ICS or allergic sensitization status was known. Dashed red lines represent normal cutoff for BAL eosinophils. Statistics represent the results of chi-square tests for (*A* and *D*) categorical data or (*B*, *C*, *E*, and *F*) D'Agostino and Pearson normality tests followed by the Kruskal-Wallis tests for continuous nonparametric data, with Dunn's correction for multiple comparisons being used $^*P < 0.05$ and $^{**}P < 0.01$.

dominated by *Haemophilus*, *Staphylococcus*, and *Streptococcus* ($P = 0.02$) (Table 2; Figure E13A). No differences were found between cluster membership and the distribution of viruses, with rhinovirus being prevalent across all groups (Figure E13B).

Pathophysiological clusters and clinical phenotypes. The association of cluster membership and clinical phenotypes is shown in Table 3 and Figure E14. There was a highly significant difference in cluster allocation between the RSW and NWRD groups, in that children with RSW were approximately equally distributed across the pathophysiological clusters, whereas the majority of children with NWRD (60%) were assigned to cluster 4; not a single child with NWRD was assigned to cluster 1. Among

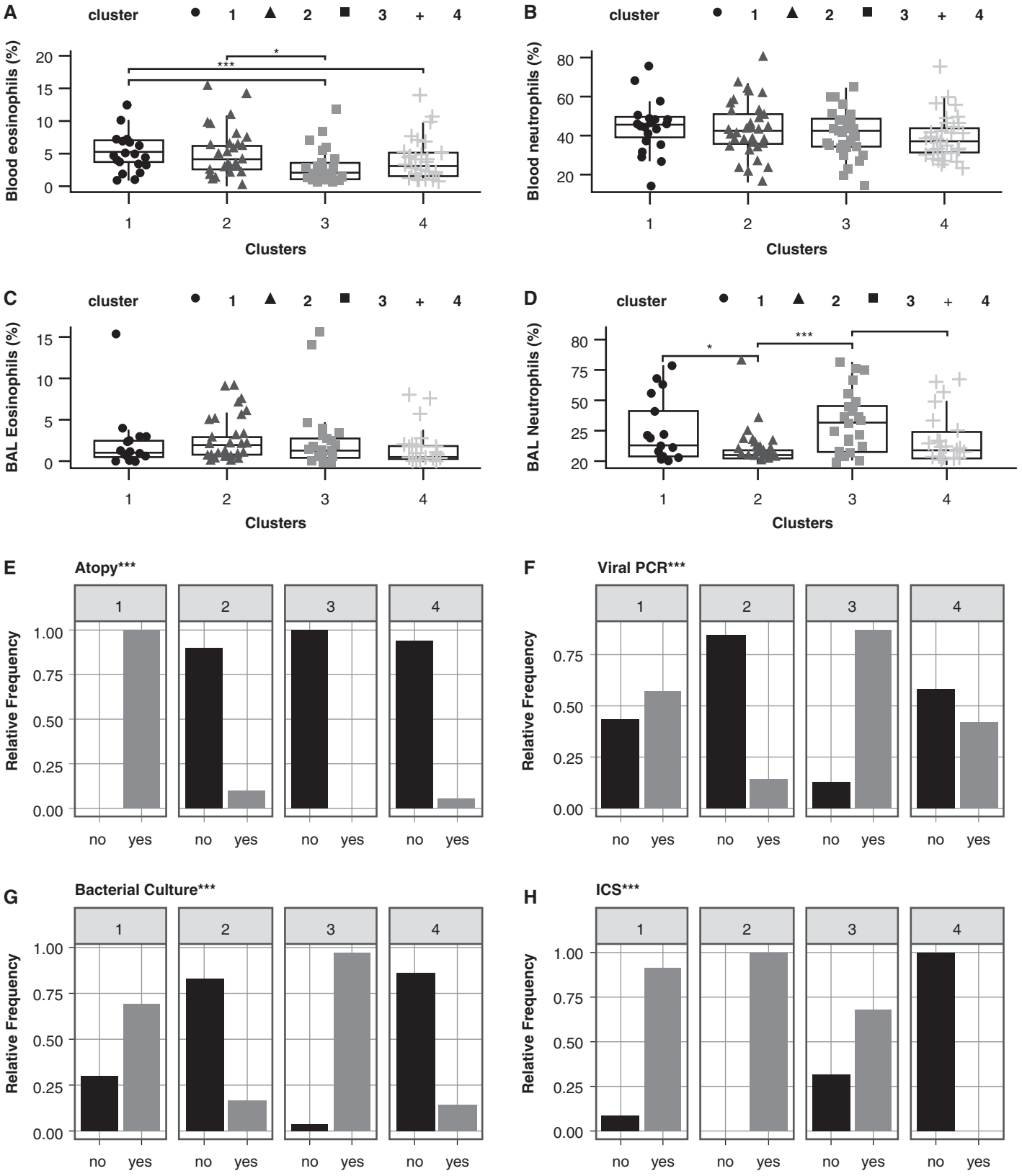

Figure 4. Cluster characteristics and cluster distribution in preschool children with severe wheeze. (*A–D*) Within-cluster distribution of BAL-sample eosinophils (percentage), BAL-sample neutrophils (percentage), peripheral blood eosinophils (percentage), and peripheral blood neutrophils (percentage). Data were analyzed by using *t* tests. Boxplots represent the 25th and 75th percentiles and the median. (*E–H*) Within-cluster distribution of atopy, use of ICS, positive bacteriology results, and positive viral PCR results. Data were analyzed by using Fisher exact tests. Bar charts represent the relative frequency of each category. $^{*}P < 0.05$ and $^{***}P < 0.001$. ICS = inhaled corticosteroids.

Table 2. Demographic, Clinical Characteristics, Infection, and Cluster Features of Pathophysiological Clusters

	Cluster 1 (*N* = 24)	Cluster 2 (*N* = 42)	Cluster 3 (*N* = 31)	Cluster 4 (*N* = 37)	*P* Value
Demographics					
Sex, M, *n* (%)	11/24 (45.8)	28/42 (66.7)	20/31 (64.5)	21/37 (56.8)	0.363
Age, mo, mean (SD)	45.76 (17.74)	38.06 (15.32)	30.90 (13.86)	30.47 (13.98)	<0.001
Family history of asthma, *n* (%)	10/19 (52.6)	23/31 (74.2)	14/23 (60.9)	18/27 (66.7)	0.446
Current exposure to pets, *n* (%)	5/19 (26.3)	8/33 (24.2)	11/24 (45.8)	11/28 (39.3)	0.301
Exposure to pets during pregnancy, *n* (%)	2/12 (16.7)	8/19 (42.1)	6/18 (33.3)	9/20 (45.0)	0.409
Current household smoking, *n* (%)	6/22 (27.3)	11/34 (32.4)	6/25 (24.0)	7/29 (24.1)	0.879
Passive smoking, *n* (%)	6/22 (27.3)	11/35 (31.4)	9/26 (34.6)	8/29 (27.6)	0.946
Gestational age, mean (SD)	38.850 (1.541)	39.159 (1.554)	38.703 (1.412)	39.171 (1.458)	0.563
Birth weight, mean (SD)	3.135 (0.616)	3.221 (0.687)	3.254 (0.441)	3.287 (0.516)	0.843
Height, mean (SD)	99.000 (11.429)	96.657 (13.461)	92.284 (10.765)	90.152 (8.795)	0.021
Weight, mean (SD)	15.637 (4.162)	15.453 (3.597)	13.887 (2.721)	13.342 (2.412)	0.023
Duration of breastfeeding, mo, mean (SD)	7.625 (6.739)	4.471 (4.336)	5.125 (5.524)	4.807 (6.230)	0.617
Urban environment, *n* (%)	8/10 (80.0)	17/22 (77.3)	15/18 (83.3)	18/23 (78.3)	0.977
Clinical characteristics					
Wheeze ever, *n* (%)	20/20 (100.0)	36/37 (97.3)	21/23 (91.3)	19/26 (73.1)	0.004
Current wheeze, *n* (%)	24/24 (100.0)	38/42 (90.5)	22/31 (71.0)	20/37 (54.1)	<0.001
More than three episodes of wheeze ever, *n* (%)	17/17 (100.0)	25/27 (92.6)	17/21 (81.0)	15/22 (68.2)	0.023
More than three episodes of wheeze in the past 6 mo, *n* (%)	13/13 (100.0)	21/23 (91.3)	15/19 (78.9)	10/20 (50.0)	0.001
Persistent cough, *n* (%)	7/24 (29.2)	12/42 (28.6)	13/31 (41.9)	19/37 (51.4)	0.153
Upper airway problem and noisy breathing, *n* (%)	1/24 (4.2)	2/42 (4.8)	6/31 (19.4)	5/37 (13.5)	0.162
Recurrent chest infections, *n* (%)	5/24 (20.8)	12/42 (28.6)	10/31 (32.3)	12/37 (32.4)	0.767
Hospitalizations, *n* (%)	7/12 (58.3)	8/22 (36.4)	7/15 (46.7)	4/15 (26.7)	0.469
Number of hospital admissions, mean (SD)	16.700 (9.673)	6.435 (5.053)	16.214 (26.516)	3.429 (3.056)	0.025
Number of hospital admissions/mo, mean (SD)	0.468 (0.312)	0.206 (0.182)	0.457 (0.605)	0.156 (0.127)	0.029
Abnormal pH result, *n* (%)	6/22 (27.3)	9/31 (29.0)	5/22 (22.7)	6/29 (20.7)	0.919
Medication, *n* (%)					
Salbutamol (ever)	24/24 (100.0)	40/40 (100.0)	25/28 (89.3)	27/29 (93.1)	0.054
Montelukast (ever)	17/17 (100.0)	37/38 (97.4)	20/25 (80.0)	17/25 (68.0)	0.001
OCS ever	11/11 (100.0)	31/33 (93.9)	18/24 (75.0)	18/24 (75.0)	0.057
Oral antibiotics ever	13/13 (100.0)	30/30 (100.0)	20/22 (90.9)	24/26 (92.3)	0.236
Infection, *n* (%)					
Rhinovirus vs. others	9/13 (69.2)	3/6 (50.0)	18/26 (69.2)	10/14 (71.4)	0.807
Staphylococcus, Haemophilus, and *Streptococcus* vs. others	7/16 (43.8)	6/7 (85.7)	25/30 (83.3)	3/5 (60.0)	0.023
Moraxella vs. others	11/17 (64.7)	1/7 (14.3)	9/30 (30.0)	1/5 (20.0)	0.043
Sensitization					
Number of allergens, mean (SD)	2.417 (2.586)	0.278 (0.849)	0.000 (0.000)	0.143 (0.692)	<0.001
Grasses, *n* (%)	8/24 (33.3)	1/36 (2.8)	0/22 (0.0)	0/35 (0.0)	<0.001
Cat, *n* (%)	7/24 (29.2)	1/36 (2.8)	0/22 (0.0)	0/35 (0.0)	<0.001
Dog, *n* (%)	7/24 (29.2)	1/36 (2.8)	0/22 (0.0)	0/35 (0.0)	<0.001
HDM, *n* (%)	15/24 (62.5)	3/36 (8.3)	0/22 (0.0)	0/35 (0.0)	<0.001
Aspergillus, *n* (%)	3/24 (12.5)	1/42 (2.4)	0/31 (0.0)	0/37 (0.0)	0.026
Peanut, *n* (%)	3/24 (12.5)	0/42 (0.0)	0/31 (0.0)	1/37 (2.7)	0.019
Milk, *n* (%)	5/24 (20.8)	1/42 (2.8)	0/31 (0.0)	2/37 (5.4)	0.008
Egg white, *n* (%)	2/24 (8.3)	0/42 (0.0)	0/31 (0.0)	1/37 (2.7)	0.084
Egg yolk, *n* (%)	1/24 (4.2)	0/42 (0.0)	0/31 (0.0)	0/37 (0.0)	0.179
A. alternata, *n* (%)	1/24 (4.2)	0/42 (0.0)	0/31 (0.0)	0/37 (0.0)	0.179
Cluster features					
BAL-sample eosinophils, %, mean (SD)	2.16 (3.62)	2.46 (2.50)	2.55 (3.92)	1.41 (2.10)	0.441
BAL-sample neutrophils, %, mean (SD)	25.09 (25.92)	9.44 (13.89)	31.69 (25.11)	18.23 (20.55)	0.001
Blood eosinophil count, ×109/ml, mean (SD)	0.542 (0.439)	0.500 (0.410)	0.466 (0.394)	0.385 (0.344)	0.470
Blood neutrophil count, ×109/ml, mean (SD)	4.629 (2.006)	4.564 (2.125)	5.114 (2.577)	5.139 (2.391)	0.628
Blood eosinophils, %, mean (SD)	5.54 (2.86)	4.85 (3.47)	2.87 (2.57)	3.82 (3.14)	0.008
Blood neutrophils, %, mean (SD)	44.57 (12.63)	43.17 (13.42)	42.11 (11.17)	39.15 (11.30)	0.356
Positive BAL-sample bacterial culture (yes), *n* (%)	16/23 (69.6)	7/41 (17.1)	30/31 (96.8)	5/36 (13.9)	2.50×10^{-16}
Positive BAL-sample viral PCR result (yes), *n* (%)	13/23 (56.5)	6/40 (15.0)	26/30 (86.7)	14/33 (42.4)	1.27×10^{-8}
Allergic sensitization (yes), *n* (%)	24/24 (100.0)	4/39 (10.3)	0/25 (0.0)	2/36 (5.6)	9.49×10^{-21}
Inhaled corticosteroids (yes), *n* (%)	22/24 (91.7)	40/40 (100.0)	21/31 (67.7)	0/36 (0.0)	1.46×10^{-25}

Definition of abbreviations: *A. alternata* = *Alternaria alternata*; HDM = house dust mite; OCS = oral corticosteroids.
Counts and percentages (in parentheses) were reported for categorical variables, and means and SDs (in parentheses) were reported for continuous variables. Differences were assessed by using Fisher exact tests or one-way ANOVA as appropriate.

Table 3. Cluster Membership for 104 Children in the RSW Group, 30 Children in the NWRD Group, and 74 Children with RSW Who Could Be Accurately Assigned to Wheeze Phenotypes (MTW and EVW)

Cluster Membership [*n* (%)]	RSW (*N* = *104*)	NWRD (*N* = *30*)	*P*	MTW (*N* = *44*)	EVW (*N* = *30*)	*P*
Cluster 1	24 (23.1)	0 (0.0)	**<0.001**	8 (18.2)	8 (26.7)	0.360
Cluster 2	38 (36.5)	3 (10.0)		12 (27.3)	12 (40.0)	
Cluster 3	22 (21.2)	9 (30.0)		13 (29.5)	5 (16.7)	
Cluster 4	20 (19.2)	18 (60.0)		11 (25.0)	5 (16.7)	

Definition of abbreviations: EVW = episodic viral wheeze; MTW = multiple-trigger wheeze; NWRD = nonwheezing respiratory disease; RSW = recurrent severe wheeze.
Bold represents statistically significant *P* values.

children with RSW, there was no difference in cluster membership between those with EVW and those with MTW. In the NWRD group, there were clinically meaningful differences in cluster membership among children with different diagnoses (Table E6).

Discussion

To our knowledge, this report includes the largest cohort of preschoolers with severe respiratory symptoms undergoing elective bronchoscopy, with information about atopy, prescribed treatments, and lower airway inflammation and infection, to ascertain the relationship between clinical diagnostic labels and airway pathology. As a group, preschoolers with severe wheeze had higher blood eosinophil counts and percentages but had similar BAL-sample eosinophil and neutrophil percentages compared with children undergoing bronchoscopy for NWRD. Among those with severe wheeze, different wheeze phenotypes (EVW or MTW) did not have differing lower airway pathology. Having found no distinctions in lower airway inflammation and infection between children with severe wheezing and children with nonwheezing disease or among clinical wheeze phenotypes, we undertook an unbiased analysis to determine whether distinct pathophysiological clusters could be identified without prespecified diagnostic labels. Four stable clusters were identified (atopic, nonatopic with a low infection rate and high use of ICS, nonatopic with a high infection rate, and nonatopic with a low infection rate and no use of ICS). We observed striking differences in cluster allocation between those with severe wheeze and control subjects with NWRD. Those with severe wheeze were approximately equally distributed across the pathophysiological clusters, whereas in marked contrast, the majority (60%) of children without wheezing were assigned to the nonatopic cluster with a low infection rate, and not a single child in the NWRD group was assigned to the atopic cluster. Among those with severe wheeze, there was no difference in cluster membership between those with EVW and those with MTW, suggesting that these clinical labels bear little or no relationship to the underlying pathology. The variables that discriminated between the clusters included blood eosinophil counts and percentages, BAL-sample neutrophil percentages, allergic sensitization, and the pattern of airway bacterial infection: *Moraxella* versus *Staphylococcus* or *Haemophilus* or *Streptococcus*.

Limitations and Strengths

It would be preferable to probe the data on lower airway inflammation and infection among those with wheeze who are not prescribed ICS, as this therapy may impact the results. It is unlikely that such data will be available, as the majority of those undergoing clinically indicated bronchoscopy will be prescribed ICS. All children with RSW in our study had severe, recurrent wheezing (median of six hospitalizations) and most had commenced treatment with ICS. To mitigate this, we used prescription of ICS as one of the dimensions in clustering. We accept that adherence to treatment with ICS and the lower airway deposition in this age group were unknown. Moreover, because allergic sensitization predicts the response to ICS and these children with RSW underwent bronchoscopy because ICS failed to control their symptoms, they may be a less atopic cohort (27).

Clearly, our cohort is not representative of preschool children with wheeze from the general population, and our findings are not generalizable to the overall population but may be applicable to patients with severe disease who have the most adverse outcomes. We also acknowledge that replication in a validation cohort would be desirable; however, such studies are limited by the availability of bronchoscopy and BAL data.

We acknowledge that BAL samples were analyzed by using bacterial cultures rather than the microbiome. However, we captured key bacterial species previously associated with childhood wheezing and asthma and used methodologies accessible to practicing pediatricians. Given the very low numbers of children who were nonwhite, we were unable to undertake assessments of findings according to ethnicity, and our results are not generalizable to other ethnicities.

Our data-driven approach is exploratory and hypothesis-generating, and the cross-sectional nature of our study limits the ability to address causation. It is uncertain whether these clusters will be stable over time, in terms of either the inflammatory phenotypes or the infection phenotypes. However, the clusters we identified are intuitively correct (i.e., have face validity), and the observed differences in cluster allocation between those with severe wheeze and control subjects with NWRD and among children with different clinical diagnoses in the NWRD group provide evidence of content validity. Importantly, individual children were assigned to clusters with a high degree of certainty. We acknowledge the computational complexity of this analysis but wish to emphasize that this is

not a "black-box" or "data-mining" approach; the analysis was informed by the existing knowledge. Thus, the identified clusters can be considered as outcomes in their own right.

Interpretation

Data-driven methodologies have been used to disaggregate school-aged asthma (28). In most studies, individuals with severe asthma were present in each cluster (29–33). We found the same in preschool wheezing: children with severe disease clustered into distinct pathophysiological groups. We confirmed that the majority of children with RSW (>70%) are nonatopic, in concurrence with a cluster analysis of BAL samples from children aged 1–16 years, which described a nonatopic, neutrophilic, steroid-refractory preschool wheeze cluster (34). A recent study showed that school-aged severe asthma is more similar to adult severe asthma than to severe preschool wheeze (35). Taken together, these findings suggest that the lower airway inflammation pathways of RSW relate mostly to eosinophils in older children and neutrophils in younger children.

Half of study participants had a positive BAL-sample bacterial culture result, a proportion similar to that previously reported (36). A higher proportion of children with RSW than with NWRD had rhinovirus, which is again consistent with studies of the general population (36, 37). We extended these findings and demonstrated that different bacterial species are associated with different pathophysiological clusters: cluster 1 (atopic, high use of ICS) had *Moraxella* predominance, whereas the predominant bacteria identified in cluster 3 included *Haemophilus*, *Streptococcus*, and *Staphylococcus*.

We previously demonstrated, using nonculture techniques, *Moraxella* predominance in the lower airway microbiome of those with wheeze associated with neutrophilia (38) and have now confirmed an association between a positive BAL-sample bacterial culture result and neutrophilia during stable disease, regardless of sensitization status or prescription of ICS, suggesting that targeted antibiotic treatment may be beneficial. There are several potential explanations for the association among lower airway bacterial colonization, neutrophilia, and use of ICS. First, early-life bacterial colonization may be the primary driver for wheeze, and neonatal hypopharyngeal colonization with *Moraxella* has been shown to predict wheeze development (37). Second, bacterial infection and/or neutrophilia may occur secondary to use of ICS. In adults with neutrophilic obstructive airway disease, ICS are associated with an increased risk of bacterial infection (39, 40) and prolong neutrophil survival (41). Third, there may be a subgroup of individuals with wheeze who are susceptible to specific pathogens (e.g., *Moraxella*), leading to neutrophilia and chronic infection failing to resolve because of ICS. Mechanistic studies and prospective interventional studies investigating the relationship between use of ICS, lower airway neutrophilia, and bacterial infection are needed to help decipher their interrelationship.

The current management of RSW focuses on treating the clinical diagnosis rather than the pathological mechanism leading to symptoms in an individual patient. Despite widespread acceptance, our data suggest that clinical phenotyping into MTW and EVW may be an unreliable classification scheme. Our data may serve as a foundation for alternative stratification in future interventional therapeutic trials and may facilitate the analysis of response profiles for different treatments. Management strategies need modification with objective biomarkers that predict the response to different treatments.

Important aspects for delivering a personalized approach include understanding endotypes that give rise to symptoms in an individual and having biomarkers of these mechanisms to deploy mechanism-based treatments (4). Our data-driven analyses identified biomarkers (sensitization and peripheral eosinophilia) and an atopic cluster that is similar in size (approximately one-fourth of preschool children with wheeze) to a subgroup within the individualized therapy for persistent asthma in young children (INFANT) study (a randomized clinical trial in preschoolers) in whom treatment with daily ICS was beneficial (27). On the basis of the known mechanisms of action of ICS (42), this suggests that treatment with ICS would be appropriate for children in cluster 1 in our study. Our data indirectly confirm that preschool children with wheezing who are considered for treatment with ICS should have phenotyping with aeroallergen sensitization and blood eosinophil counts and percentages (27, 43). Because *Moraxella* dominates infection in these children, using induced sputum to identify bacterial infection and appropriate antibiotics may be beneficial, particularly for those with a suboptimal response to ICS.

Biomarkers and treatments for the majority of preschool children with wheeze and without sensitization or blood eosinophilia remain elusive. However, our analyses provide pointers for future potential stratified approaches for this group. Pathophysiological cluster 3 (nonatopic, high infection rate) was characterized by the highest prevalence of bacterial and viral infection (96.8% and 89.7%, respectively), the highest BAL-sample neutrophil percentages, and *Haemophilus, Staphylococcus* and *Streptococcus* detection. Future intervention studies should test whether these children would benefit from targeted antibiotic therapy, as it seems reasonable to treat these children on the basis of induced sputum bacteriology results (a feasible and safe noninvasive technique to assess infection in young children [21, 44, 45]).

In our study, the majority of children with no lower airway symptoms were in cluster 4 and had no evidence of sensitization, airway inflammation, or infection. However, 20% of those with severe wheeze were also assigned to this cluster. If airway obstruction is contributing to symptoms in this group and can be objectively measured, a potential treatment that may be of benefit is the use of as-required bronchodilators and/or muscarinic antagonists.

In conclusion, we have demonstrated that severe preschool wheezing encompasses a range of conditions with distinct pathophysiologies and that novel pathophysiological clusters can be revealed by data-driven approaches taking advantage of the data on lower airway inflammation and infection collected from patients undergoing clinical bronchoscopy. Our results suggest that a change in the taxonomy of childhood wheezing disorders is needed to reflect the underlying mechanisms. Until we fully understand the subtype mechanisms, we should consider RSW a "preschool wheezing spectrum disorder," with sensitization, peripheral eosinophilia, and lower airway microbiology being used as potential biomarkers to stratify treatment. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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