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

Polly F. M. Robinson1,2, Sara Fontanella1*, Sachin Ananth1,2, Aldara Martin Alonso1, James Cook1,2, Daphne Kaya-de Vries1,2, Luisa Polo Silveira1,2, Lisa Gregory1, Clare Lloyd1, Louise Fleming1,2, Andrew Bush1,2, Adnan Custovic1‡, and Sejal Saglani1,2‡

1National Heart and Lung Institute, Imperial College London, London, United Kingdom; and 2Paediatric Respiratory Department, Royal Brompton and Harefield Hospitals, London, United Kingdom

© Copyright 2021 by the American Thoracic Society

Am J Respir Crit Care Med Vol 204, Iss 5, pp 523–535, Sep 1, 2021

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

(Received in original form September 29, 2020; accepted in final form May 5, 2021)

*These authors contributed equally to this work.

†These authors contributed equally to this work.

‡A.C. is Associate Editor of AJRCCM. His participation complies with American Thoracic Society requirements for recusal from review and decisions for authored works.

This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License 4.0. For commercial usage and reprints, please e-mail Diane Gern.

Supported by the British Lung Foundation grant RG14-12. Unsupervised analysis was funded in part by the Medical Research Council grant MR/S025340/1. P.F.M.R. was a National Institute for Health Research academic clinical fellow and had a National Heart and Lung Institute master’s scholarship. L.F. is an Asthma UK senior clinical fellow, C.L. is a Wellcome senior fellow, A.B. is a National Institute for Health Research senior investigator, and S.S. was a National Institute for Health Research career development fellow (CDF-2014-07-019).

Author Contributions: P.F.M.R., S.A., J.C., D.K.-d.V., and L.P.S. collected patient data and samples; P.F.M.R., S.A., A.M.A., D.K.-d.V., L.P.S., and L.G. analyzed samples; P.F.M.R., S.F., S.A., A.M.A., D.K.-d.V., and L.P.S. analyzed data. S.S. supervised data collection. P.F.M.R. and S.F. wrote the manuscript. L.F. and A.B. contributed patients, data interpretation, and manuscript editing. L.G. and C.L. contributed to data interpretation and manuscript editing. S.S. and A.C. conceived the study design, supervised data collection and analysis, and wrote and edited the manuscript. All authors revised and approved the final version to be published.

Am J Respir Crit Care Med Vol 204, Iss 5, pp 523–535, Sep 1, 2021
Copyright © 2021 by the American Thoracic Society
Originally Published in Press as DOI: 10.1164/rccm.202009-3696OC on May 5, 2021
Internet address: www.atsjournals.org
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 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 \( \geq 0.35 \) 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.
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 $\sim$80% and 95% of these samples were evaluated 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 ($\sim$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, and Infection, and Cluster Membership

| Demographics | RSW (N = 105) | NWRD (N = 31) | P | MTW (N = 44) | EVW (N = 30) | No Phenotype (N = 31) |
|--------------|----------------|----------------|-----|----------------|---------------|------------------------|
| Sex, M, n (%) | 66 (63)        | 15 (48)        | 0.21 | 30 (68)        | 17 (57)       | 19 (61.3)              |
| Age, mo, x (range) | 34.8 (13–70) | 33.1 (14–64) | 0.39 | 30.5 (13–70) | 34.9 (14–69) | 38.8 (14–69) |
| Weight, kg, x (range) | 14.3 (0.9–26) | 14.0 (0.9–22) | 0.35 | 14.2 (0.9–22 [40]) | 14.3 (12–24 [26]) | 14.4 (12–21 [16]) |
| Height, cm, x (range) | 94.3 (66–121 [83]) | 91.8 (73–113 [23]) | 0.42 | 89.8 (56–117 [38]) | 94.3 (81–115 [27]) | 97 (83–121 [16]) |
| 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 (7)                  |
| Mixed/other, n (%) | 8 (13)         | 1 (9)          | —   | 3 (9)          | 3 (14)        | 2 (33)                 |
| Perinatal factors |               |                |      |                |               |                       |
| Gestation, wk, x (range) | 39 (36–42 [93]) | 40 (36–41 [23]) | 0.68 | 39 ± 1 (36–42 [44]) | 39 ± 6 (36–42 [29]) | 39 (36–42 [20]) |
| Vaginal delivery, n (%) | 51/77 (66) | 6/17 (35) | 0.03 | 29/37 (78) | 17/27 (63) | 5/13 (38) |
| Birth weight, kg, x (range) | 3.2 (1.4–4.3) | 3.4 (1.4–4.1 [16]) | 0.74 | 3.2 (2.2–4.3 [40]) | 3.5 (1.4–4.2 [27]) | 2.9 (1.9–3.6 [14]) |
| Oxygen required, n (%) | 3/75 (4) | 1/13 (8) | 0.48 | 2/45 (5) | 0/26 (0) | 1/7 (14) |
| Ventilation, n (%) | 2/76 (3) | 2/14 (14) | 0.11 | 1/43 (2) | 0/26 (0) | 1/7 (14) |
| Breastfeeding, n (%) | 49/76 (64) | 9/13 (63) | 0.09 | 24/35 (69) | 17/26 (63) | 6/15 (33) |
| Breastfeeding duration, mo, x (range) | 4 (0–24 [48]) | 4 (0–18 [9]) | 0.38 | 3 (0–12 [25]) | 6 (0–24 [16]) | 1 (3–9 [5]) |
| Clinical features |               |                |      |                |               |                       |
| Allergic sensitization, n (%) | 30/100 (30) | 1/23 (4) | 0.008 | 12/42 (29) | 9/28 (32) | 9/30 (30) |
| TRACK score, x (range) | 17 (4–26 [15]) | N/A | N/A | 16 (4–23 [9]) | 23 (5–26 [5]) | 0 (0–0) |
| PACQLQ score, x (range) | 4.1 (1–7 [47]) | N/A | N/A | 3.8 (1–7 [31]) | 5.3 (1–7 [16]) | 0 (0–0) |
| Hospitalizations for wheeze, x (range) | 6 (0–100 [59]) | N/A | N/A | 6 (0–100 [31]) | 6 (0–21 [22]) | 4 (2–6 [2]) |
| Birthweight, kg, x (range) | 3.7 (1.4–4.4) | 3.1 (1.1–3.9) | 0.03 | 3.5 (2.2–4.3) | 3.5 (2.2–4.3) | 2.9 (1.9–3.6) |
| Vaginal delivery, n (%) | 52 (59) | 10 (22) | 0.002 | 28 (22) | 9 (21) | 2 (9) |

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; 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; 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 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. ***\( P < 0.001 \).
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 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 negative bacterial culture results and allergic sensitization (*n* = 13), gray circles represent children with RSW with negative bacterial culture results and no allergic sensitization (*n* = 8), black squares represent children with RSW with positive bacterial culture results and allergic sensitization (*n* = 8), and gray squares represent children with RSW with positive 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.
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

| Demographics | Cluster 1 (N = 24) | Cluster 2 (N = 42) | Cluster 3 (N = 31) | Cluster 4 (N = 37) | P Value |
|---------------|--------------------|--------------------|--------------------|--------------------|---------|
| 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 |
| Demographic, Clinical Characteristics, Infection, and Cluster Features | 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 |
| Obesity, 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 | 20/20 (100.0) | 36/37 (97.3) | 21/23 (91.3) | 19/26 (73.1) | 0.004 |
| Persistent cough, n (%) | 12/24 (50.0) | 12/24 (50.0) | 12/24 (50.0) | 12/24 (50.0) | 0.162 |
| Recurrent chest infections, n (%) | 5/24 (20.8) | 12/24 (50.0) | 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) | 67.000 (9.673) | 64.35 (5.053) | 61.214 (26.516) | 3.294 (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 (%) | 22/24 (91.7) | 40/40 (100.0) | 36/37 (97.3) | 21/23 (91.3) | 0.001 |
| Salbutamol (ever) | 7/24 (29.2) | 12/24 (50.0) | 9/13 (69.2) | 9/24 (37.5) | 0.153 |
| 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 (%) | 9/13 (69.2) | 3/6 (50.0) | 18/26 (69.2) | 10/14 (71.4) | 0.807 |
| Rhinovirus vs. others | 7/16 (43.8) | 8/27 (30.0) | 25/30 (83.3) | 3/5 (60.0) | 0.023 |
| Streptococcus vs. others | 11/17 (64.7) | 1/7 (14.3) | 9/30 (30.0) | 1/5 (20.0) | 0.043 |

**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.
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 presupposed 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

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 | 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.
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 intervention 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 microbiology 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 pathophysiology and 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.
Reference
38. Robinson PFM, Pattaroni C, Cook J, Gregory L, Alonso AM, Fleming LJ, et al. Lower airway microbiota associates with inflammatory phenotype in severe preschool wheeze. J Allergy Clin Immunol 2019;143:1607–1610, e3.

39. Contoli M, Pauletti A, Rossi MR, Spanevello A, Casolari P, Marcellini A, et al. Long-term effects of inhaled corticosteroids on sputum bacterial and viral loads in COPD. Eur Respir J 2017;50:1700451.

40. Singanayagam A, Glanville N, Girkin JL, Ching YM, Marcellini A, Porter JD, et al. Corticosteroid suppression of antiviral immunity increases bacterial loads and mucus production in COPD exacerbations. Nat Commun 2018;9:2229.

41. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C. How glucocorticoids affect the neutrophil life. Int J Mol Sci 2018;19:4090.

42. Raissy HH, Kelly HW, Harkins M, Szefler SJ. Inhaled corticosteroids in lung diseases. Am J Respir Crit Care Med 2013;187:798–803.

43. Sonntag HJ, Filippi S, Papis S, Custovic A. Blood biomarkers of sensitization and asthma. Front Pediatr 2019;7:251.

44. Ronchetti K, Tame JD, Paisey C, Thia LP, Doull I, Howe R, et al. The CF-Sputum Induction Trial (CF-SpIT) to assess lower airway bacterial sampling in young children with cystic fibrosis: a prospective internally controlled interventional trial. Lancet Respir Med 2018;6:461–471.

45. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005;365:130–134.