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Severe Bronchiolitis Profiles and Risk of Developing Recurrent Wheezing by Age 3 Years

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Abstract

Background. A better understanding of bronchiolitis heterogeneity may help clarify its relationship with the development of recurrent wheezing and asthma.

Objectives. To identify severe bronchiolitis profiles by a clustering approach, and to investigate for the first time their association with allergy/inflammatory biomarkers; nasopharyngeal microbiota; and development of recurrent wheezing by age 3 years.

Methods. We analyzed data from a prospective, 17-center U.S. cohort study of 921 infants (age <1 year) hospitalized with bronchiolitis (2011-2014 winters) with post-hospitalization follow-up. Severe bronchiolitis profiles at baseline (hospitalization) were determined by latent class analysis, based on clinical factors and viral etiology. Blood biomarkers and nasopharyngeal microbiota profiles were determined using samples collected within 24h of hospitalization. Recurrent wheezing by age 3 years was defined based on parental report of breathing problem episodes post-discharge.

Results. Three severe bronchiolitis profiles were identified: profile A (15%), characterized by history of breathing problems/eczema during infancy and non-RSV (mostly rhinovirus) infection; profile B (49%) with the largest probability of RSV infection and which resembled classic RSV-bronchiolitis; and profile C (36%), the most severely ill group. Profile A infants had higher eosinophil counts, higher cathelicidin levels, and elevated proportions of *Haemophilus*-dominant or *Moraxella*-dominant microbiota profile. Compared to profile B, we observed significantly increased risk of developing recurrent wheezing in children with profile A (hazard ratio 2.64; 95% CI 1.90-3.68), and, to a lesser extent, with profile C (1.51; 1.14-2.01).

Conclusion. Although longer follow-up is needed, our results may help identify, among children hospitalized for bronchiolitis, subgroups with particularly elevated risk of developing asthma.

Abstract word count: 253

Key messages

- In a large cohort of infants hospitalized for bronchiolitis, the authors used a clustering approach to identify three distinct clinical profiles of severe bronchiolitis
- Significant differences across the profiles for allergy and inflammatory markers and nasopharyngeal microbiota were found, suggesting a biological basis for the observed heterogeneity
- The severe bronchiolitis profiles were differentially associated with development of recurrent wheezing by age 3 years

Capsule summary

Severe bronchiolitis profiles identified by a clustering approach were differentially associated with allergy and inflammatory biomarkers, nasopharyngeal microbiota profiles, and the risk of developing recurrent wheezing by age 3 years.

Key words: Respiratory infections, Cluster Analysis, Respiratory Syncytial Virus, Rhinovirus, Asthma

Abbreviations:

CI: confidence interval

ED: emergency department

ICS: inhaled corticosteroids

ICU: intensive care unit

IgE: Immunoglobulin E

HR: hazard ratio

MARC-35: 35th Multicenter Airway Research Collaboration

25(OH)D: 25-Hydroxyvitamin D

OR: odds ratio

RSV: respiratory syncytial virus

INTRODUCTION

Early life viral respiratory infections are associated with increased risk of developing recurrent wheezing and asthma.¹⁻³ Bronchiolitis is the most common acute lower respiratory tract infection in infancy.^{4,5} Although most children with bronchiolitis experience mild to moderate illness, symptoms can be severe enough to require hospitalization.⁶ Severe bronchiolitis is a strong predictor of long-term respiratory morbidity; indeed, 30-40% of infants hospitalized for bronchiolitis will develop recurrent wheezing or asthma.^{7,8} However, the factors predicting which infants will develop chronic respiratory illness remain incompletely characterized.

Bronchiolitis is generally considered a single disease, however, recent studies have underlined its heterogeneity.^{9,10} Beyond variability in the clinical course of the disease, patients with bronchiolitis are heterogeneous with regard to viral etiology and clinical history.^{9,11} Respiratory infections with RSV and rhinovirus, the most commonly identified pathogens in bronchiolitis, have both been associated with later development of recurrent wheezing and asthma, although stronger associations have been reported for those with rhinovirus.^{1,2,7,8,12,13} Moreover, among patients with bronchiolitis or early life wheezing illnesses, rhinovirus infection is most often seen in children with clinical histories suggesting susceptibility to asthma (eg, history of wheezing, sensitization).^{14,15}

Using a statistical clustering approach in multicenter cohorts of US and Finnish children hospitalized with bronchiolitis, we recently identified distinct severe bronchiolitis profiles.⁹ The profiles differed according to prior history of wheezing/eczema, wheezing during acute infection, levels of acute severity and viral etiology. Interestingly, the results suggested the existence of a

subgroup of children presenting with early signs of asthma during a severe bronchiolitis episode. However, respiratory outcomes after the severe bronchiolitis episode were not examined in this previous cross-sectional analysis.

The observed bronchiolitis heterogeneity may reflect the existence of several disease entities with specific pathobiology (endotypes), and varying propensity for later asthma development.¹⁰ There is increasing evidence for a complex interplay between viral pathogens, host immune response and respiratory microbiota in the pathogenesis and severity of acute respiratory infections, as well as their link with asthma-related outcomes.¹⁶⁻¹⁹ Examining the heterogeneity of bronchiolitis in relation to allergy markers, immune response parameters, nasopharyngeal microbiota, as well as long-term respiratory outcomes may thus provide insight into asthma susceptibility pathways. However, to date, no study has investigated the association of severe bronchiolitis profiles defined by a clustering approach with objectively measured biomarkers and long-term respiratory outcomes.

The MARC-35 study (35th Multicenter Airway Research Collaboration) is an ongoing, prospective multicenter cohort study of US infants (age <1 year) hospitalized with bronchiolitis and followed-up after hospitalization. In the current study, we aimed to identify severe bronchiolitis profiles by a clustering approach, and to investigate for the first time their association with (1) allergy, inflammatory and immune response biomarkers; (2) nasopharyngeal microbiota profiles; and (3) the risk of developing recurrent wheezing by age 3 years.

MATERIAL AND METHODS

Study design and data collection

The MARC-35 study is coordinated by the Emergency Medicine Network (<http://www.emnet-usa.org/>).¹⁸ During the 2011-2014 winter seasons (November-March), investigators at 17 sites across 14 US states enrolled 1,016 infants hospitalized with bronchiolitis (study baseline). Bronchiolitis was diagnosed by the attending physician, in accordance with the American Academy of Pediatrics guidelines.²⁰ Researchers conducted a structured interview to collect data on patients' demographic characteristics, medical history and details of the acute illness. Further clinical data were obtained from emergency department (ED) and inpatient medical records. Researchers collected blood samples and nasopharyngeal aspirates upon hospitalization, using a standardized protocol. Testing was performed for a large panel of respiratory viruses using real-time polymerase chain reaction assays, and microbiota using 16S rRNA gene sequencing (see online repository).

Among the 921 children (91%) eligible for long-term follow-up – the chronic cohort – we conducted telephone interviews with families ~1 week after hospital discharge and ~3 weeks after hospital admission, and then every 6 months from age 6 months onwards. We collected detailed data about any new breathing problem episodes, including date, duration, use of medication and whether this problem affected the child's sleep. Written informed consent was obtained from a parent or guardian of all patients included in the study. The institutional review board at each participating hospital approved the study.

Severe bronchiolitis profiles at baseline (infancy)

Severe bronchiolitis profiles were determined by latent class analysis, based on medical history, clinical course of bronchiolitis and viral etiology. Selection of variables and number of classes were performed as described earlier⁹ (see online repository). Each child was assigned to the class for which he had the highest membership probability.

Biomarkers and nasopharyngeal microbiota profiles at baseline (infancy)

Complete blood count was performed locally at each hospital. Serum allergy (total and allergen-specific IgE), vitamin D (25(OH)D), and innate immune response (cathelicidin [LL-37]) biomarkers were measured from the blood sample.¹⁷ We dichotomized total IgE level, eosinophil count and 25(OH)D level according to standard cut-offs (100 ku/L, 300 cells/ μ L and 30 ng/mL, respectively) and LL-37 level according to median value (46 ng/mL).¹⁷

To examine nasopharyngeal microbiota, we identified four distinct microbiota profiles (*Moraxella*-dominant, *Haemophilus*-dominant, *Streptococcus*-dominant, and mixed profiles) as previously described,¹⁸ by using partitioning around medoids with weighted UniFrac distance.

Respiratory outcomes by age 3 years

Recurrent wheezing by age 3 years was defined by parental report of at least 2 corticosteroid-requiring breathing problems in 6 months or at least 4 breathing problem episodes in one year that last at least one day and affect sleep.²¹ Parent-reported use of inhaled corticosteroids (ICS) or montelukast by age 3 years was also examined. Only breathing problem episodes and medication use reported post-hospital-discharge were included in the outcome definitions. Asthma by age 3 years was defined based on parental report that child was diagnosed with asthma by a doctor or other health professional.

Statistical analyses

Associations of severe bronchiolitis profiles with biomarkers and nasopharyngeal microbiota profiles at baseline were evaluated by multinomial logistic regressions. The association between severe bronchiolitis profiles at baseline and development of recurrent wheezing by age 3 years was evaluated using Kaplan-Meier survival curves and Cox models. Multivariable models adjusted for age, sex and race/ethnicity. Nasopharyngeal microbiota models were further adjusted for antibiotic and corticosteroid use. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Follow-up data were available for >80% at each follow-up interview from age 6 months to age 3 years (range: 82% to 88%). The mean age of the children at baseline was 4.1 months (SD: 3.0) and 40% were female; 50% of children were non-Hispanic blacks or Hispanics.

Severe bronchiolitis profiles

Three severe bronchiolitis profiles, labelled A-C, were identified by latent class analysis. The mean class membership probability range was 81%-84%. In Table 1, the profiles are described based on the proportion of children presenting with each characteristic (variables used for classification), according to the class they were assigned to by the latent class analysis. A summary of the profiles is presented in Figure 1, where the profiles are displayed according to their main distinctive characteristics. Further data are provided in online repository.

Profile A infants (15%) were characterized by a higher proportion of history of breathing problems (44%) and history of eczema (23%); they also differed from other profiles by the low proportion of infants with RSV infection (5%) and higher proportion of rhinovirus infection (54%). In contrast with profile A, profile B (49%) had the largest proportion of infants with RSV infection (99%) and a low proportion of infants with history of breathing problems (12%) or eczema (14%). Profile C (36%) was the most severely ill group: 60% of profile C infants had moderate-to-severe retractions at presentation (vs. 23-30% in other profiles), and 21% were hospitalized 7 days or longer (vs. 0-1% in other profiles); they also had a higher respiratory rate during hospital stay. Most profile C infants (83%) were infected with RSV.

The distribution of sociodemographic and additional clinical characteristics across profiles is presented in Table 2. Compared to other profiles, profile A infants were older and

more often Hispanic; they also more often had history of antibiotics and corticosteroid use before bronchiolitis hospitalization. The most severe group (profile C) included younger infants, and a higher proportion of infants with inadequate oral intake at ED presentation and who required ICU admission and mechanical ventilation. Regarding viral etiology, it was notable that a large proportion (46%) of profile A infants were neither infected with RSV nor rhinovirus. Other pathogens identified in >5% of profile A infants were: human metapneumovirus B (15%), adenovirus (11%), human bocavirus type 1 (10%), and parainfluenza virus type 3 (6%). For 15% of profile A infants, no pathogen was identified. Because profile B was the largest group and most resembled “classic” RSV bronchiolitis, it was used as the reference category in subsequent analyses.

Severe bronchiolitis profiles and biomarkers

Overall, no significant difference was observed in biomarkers levels between profiles B and C, but profile A differed for some biomarkers (Table 3). Total IgE level was not significantly different in profile A compared to profile B. In contrast, profile A infants had significantly higher eosinophil count than profile B infants. No difference was observed between profiles for allergic sensitization as defined by any positive value to allergen-specific IgE testing. When examining mono-sensitization (only one allergen, 15%) and multi-sensitization (two allergens or more, 4%), results suggested that profile A infants had lower odds of mono-sensitization (odds ratio: 0.56, 0.30-1.03; P=0.06), and did not differ significantly for multi-sensitization (1.34, 0.54-3.30; P=0.52) as compared to profile B infants. Profile C infants did not differ significantly from profile B infants for these specific IgE groupings (table E3, online repository).

No difference in serum 25(OH)D level was observed across the profiles. However, profile A infants had significantly higher level of serum LL-37 than infants in profiles B.

Severe bronchiolitis profiles and nasopharyngeal microbiota

Nasopharyngeal microbiota composition differed across severe bronchiolitis profiles (Table 4). In adjusted analyses, when compared to profile B, both profile A (P=0.004) and profile C (P=0.001) children more often had a *Haemophilus*-dominant microbiota profile. In addition, profile A was the group with the largest proportion of children with a *Moraxella*-dominant microbiota profile, while the proportion was the lowest in profile C children. This difference was significant when comparing profile A children to profile C children (odds ratio: 1.93, 1.08-3.45; P=0.03).

Severe bronchiolitis profiles and respiratory outcomes by age 3 years

By age 3 years, 251 children (27%) had developed recurrent wheezing overall. In profile A children, 43% had developed recurrent wheezing; in profile B, 21%; and in profile C, 29%. The Kaplan-Meier plot (Figure 2) indicates increased risk of developing recurrent wheezing by age 3 years in profile A children, and to a lower extent, in profile C children, compared to profile B. These results were confirmed in multivariable Cox proportional hazards models (Table 5): compared to profile B, we observed significantly increased risk of developing recurrent wheezing associated with profile A (hazard ratio 2.64; 95% confidence interval: 1.90-3.68, P<0.001) and with profile C (1.51; 1.14-2.01, P=0.004). We found similar associations with use of inhaled corticosteroids/montelukast by age 3 years. When examining doctor-diagnosed asthma, increased risk was also observed in profile A children compared to profile B; in contrast,

increased risk of asthma was not significantly associated with profile C. Significant differences for the risk of developing each outcome were also observed when comparing profile A children to profile C children.

Results were unchanged in sensitivity analyses (Table E4, online repository) where associations were further adjusted for daycare attendance, exposure to environmental tobacco smoke, maternal smoking during pregnancy, number of siblings at home, gestational age, delivery mode (vaginal *vs.* caesarian section), breastfeeding (from birth to age 3 months), and annual household income; or when we used a model accounting for a potential center effect (patient clustering at the hospital level) through a generalized estimating equations approach. Moreover, the association between severe bronchiolitis profiles and recurrent wheezing was not modified by age, sex, or race/ethnicity (all $P_{\text{interaction}}$ were not statistically significant).

DISCUSSION

Three distinct clinical profiles of severe bronchiolitis were identified in a large sample of infants hospitalized for bronchiolitis. Profile A was characterized by history of breathing problems/eczema during infancy and non-RSV (mostly rhinovirus) infection; profile B had the largest probability of RSV infection and resembled classic RSV-bronchiolitis; profile C was the most severely ill group. Furthermore, we showed for the first time significant differences across the profiles for biological markers and nasopharyngeal microbiota at baseline, as well as respiratory outcomes by age 3 years. Notably, compared to profile B, profile A children had a more than 2-fold increased risk of developing recurrent wheezing by age 3 years; to a lesser extent, the risk was also augmented in profile C children.

The severe bronchiolitis profiles identified by latent class analysis are consistent with earlier findings in similar US and Finnish cohorts of children hospitalized for bronchiolitis, in which 3 to 4 profiles were identified using the same clustering method.⁹ A larger number of profiles was found in the largest cohort (n=2,207), in which more heterogeneity may have been observed. In all three cohorts - including the current study - profile A, characterized by non-RSV (mostly rhinovirus) infection and history of breathing problems and eczema, was clearly identified as a separate group. The other profiles identified in the three cohorts all had a higher probability of RSV infection than profile A, and differed according to level of acute severity. Besides similarity with previous studies, the validity of the profiles is supported by the high value of mean class membership probability.

In the previous cross-sectional analysis of U.S. and Finnish children hospitalized for bronchiolitis, we hypothesized that profile A was constituted by children presenting with early signs of asthma during a severe bronchiolitis episode, but did not examine long-term respiratory outcomes.⁹ Findings from the current study provide support for this hypothesis, especially the clear, prospective association between profile A bronchiolitis and development of recurrent wheezing by age 3 years. Not all children with recurrent wheezing develop asthma. However, early (\leq age 3 years) recurrent wheezing is a strong predictor of later asthma development.²² Moreover, although a longer follow-up is needed to ultimately confirm an association with development of childhood asthma, a prospective association between profile A bronchiolitis was also observed with development of doctor-diagnosed asthma by age 3 years.

Whether viral infections have a causal role in the development of recurrent wheezing and asthma, are a sign of susceptibility to respiratory illnesses, or correspond to their first manifestations, is a long-running debate.^{1,2,23} The hypothesis that acute respiratory infections consist of distinct endotypes with different roles on the causal pathway to asthma may help clarify this important question. Profile A infants often had a clinical history suggestive of susceptibility to asthma or atopic diseases, including history of breathing problems or eczema, or prior use of corticosteroids. The hypothesis that some children with higher propensity to rhinovirus infection also have increased asthma susceptibility is consistent with a few earlier reports.¹ In particular, early signs of atopic condition have been found to precede rhinovirus-induced infections in children at high risk for asthma or allergies.¹⁴ However, this susceptibility hypothesis may not be restricted to rhinovirus. An increased risk of asthma associated with the frequency of troublesome respiratory episodes in the first 3 years of life (suggestive of potential susceptibility or exaggerated response to infections), rather than with the specific viral etiologies,

was reported in a Danish birth cohort study.²³ In addition, both RSV and rhinovirus illnesses share important susceptibility genetic variants with asthma.^{1,24} In the current study, although rhinovirus infection was markedly more common in profile A children, nearly half of them were not infected with rhinovirus; they either had other pathogens (e.g., human metapneumovirus B, adenovirus, bocavirus) or no pathogen was identified.

Profile C infants, who also had higher risk of developing recurrent wheezing (compared to profile B), were younger at bronchiolitis hospitalization and did not have a distinctive clinical history; however they were the most severely-ill group. Bronchiolitis severity has been found to increase asthma risk in a dose-response manner.²⁵ Although severe RSV infections and related inflammation have been suggested to cause long-term lung damage, evidence for a causal mechanism is lacking.² Nonetheless, the differences in the features of profile A and C, and their differential association with biomarkers and nasopharyngeal microbiota, suggest that they may be associated with recurrent wheezing through distinct pathways.

Higher blood eosinophil counts were observed in profile A children compared to the two other groups. Blood eosinophilia in childhood²⁶ or during bronchiolitis and early respiratory infection episodes^{8,27,28} has been associated with later development of recurrent wheezing or asthma. However, the exact role of eosinophils, both during acute respiratory infections and in chronic asthma, remains poorly understood.^{29,30} During acute respiratory infection, a higher number of eosinophils has been found in patients with rhinovirus infection,^{31,32} on the other hand, in RSV-induced bronchiolitis, a potential eosinopenic response to infection has been reported.^{27,28} In older children and adults with asthma, higher blood eosinophil counts have been associated with disease severity/activity and type 2 inflammation.³⁰ Our results may thus reflect

either a different immune response to infection in profile A infants or higher eosinophil counts independently from infection in this group compared to others.

In contrast with the eosinophils findings, we did not observe a significant association between severe bronchiolitis profiles and allergy markers, although higher total IgE levels on the one hand and lower odds of mono-sensitization on the other hand were suggested in profile A infants. Eosinophilia and allergic sensitization in childhood both are associated with increased asthma risk and have been suggested to have a role in at least some common causal pathway(s).³³ However, they have been independently associated with asthma and may relate to its development at different time windows.²⁶ Indeed, sensitization is highly dependent on age and is less common in infants;³⁴ its relevance as a predictor of wheezing outcomes and asthma at such an early age is disputed.^{34,35} In future work, we will study associations of severe bronchiolitis profiles in infancy with phenotypes of allergic sensitization during childhood^{36,37} and asthma to provide further insight into potential causal pathways.

We also found higher cathelicidin levels in profile A children compared to the other groups. Human cathelicidin is an innate immunity host defense peptide with immunomodulatory properties.^{17,38} Although vitamin D is one of the factors regulating cathelicidin production and activation,³⁸ the profiles did not differ with regards to 25(OH)D levels. Direct antiviral activity of cathelicidin against both RSV and rhinovirus has been reported in experimental studies;³⁹ however, lower cathelicidin levels were found in children with RSV infection compared to those with rhinovirus or other non-RSV infection in two cohorts of children hospitalized for severe bronchiolitis, including MARC-35.^{17,40,41} This is consistent with the lower cathelicidin levels found in profiles B and C compared to profile A infants. Although the exact mechanisms and

features of profile A that are driving this association are beyond the scope of our data, our results may reflect a different immune response during acute bronchiolitis in profile A children.

Nasopharyngeal microbiota also differed according to severe bronchiolitis profiles. Interestingly, while profile B and C did not differ for the investigated biomarkers, we observed distinct nasopharyngeal microbiota profiles in these groups. The *Haemophilus*-dominant microbiota profile was more common in profile C than in profile B infants, consistent with its association with increased bronchiolitis severity previously reported in MARC-35¹⁸ and with severity of RSV-induced infection in young children.⁴² Profile A was characterized by elevated proportions of children with either the *Haemophilus*-dominant or the *Moraxella*-dominant profile. High abundance of *Haemophilus* and *Moraxella* has been reported in MARC-35 infants with rhinovirus infection.⁴³ Furthermore, in a Danish birth cohort, specific bacterial colonization in the airways of neonates, including *Haemophilus influenzae* and *Moraxella catarrhalis*, was associated with later development of pneumonia or bronchiolitis,⁴⁴ and persistent wheeze and asthma.⁴⁵ In older children, *Moraxella* abundance in nasal microbiota was also associated with asthma.¹⁹ The timing and mechanisms of the interplay between viral infections, airway microbiota and host immune response, in relation to respiratory outcomes have yet to be determined.^{16,19} While our findings are consistent with previous studies reporting associations between microbiota profiles and individual characteristics of bronchiolitis (severity, viral etiology)^{18,42,43}, they question whether microbiota profiles are independently associated with each of these characteristics, or are part of the features of distinct bronchiolitis endotypes. Overall, our findings add evidence to a contribution of both virus and bacteria to the pathogenesis of acute and chronic respiratory illnesses.^{16,42}

This study had potential limitations. First, recurrent wheezing was defined using parental reports of breathing problems episodes. Although “breathing problems” were clearly described to parents during interviews with intent to identify major respiratory symptoms, such as wheezing, cough, and shortness of breath (see details in online repository), and only those episodes that lasted at least one day and affected sleep,²¹ the outcome was not confirmed by a physician. However, our results were confirmed when examining ICS or montelukast use by age 3 years, a more objective outcome; and development of doctor-diagnosed asthma by age 3 years.¹³ Second, the severe bronchiolitis profiles identified in the current study have been defined in a cohort of hospitalized infants, which only represent a small proportion of all bronchiolitis patients. It is likely that additional bronchiolitis profiles exist, including milder cases. However, it is relevant from a public health point of view to study the most severe bronchiolitis patients given that they are at high-risk for asthma development²⁵ and bronchiolitis is the most common reason for hospitalization in U.S. infants.⁶ For a more complete understanding of bronchiolitis, similar clustering approaches should be applied to other cohorts with different designs, in particular those including outpatient cases; indeed beyond having distinct clinical presentations, outpatient cases may have differences in risk factors for bronchiolitis and socio-demographic characteristics. Finally, the patients’ profile as defined based on a statistical clustering approach cannot directly be assessed in clinical settings. However, we have identified in Figure 1 the main features of each profile to help the physicians and investigators identifying patients more likely to belong to each group. Blood eosinophilia may be an additional relevant marker to identify profile A children. Longer follow-up is needed to better determine the clinical significance of our results and before suggestions can be made about a differential management of children with severe bronchiolitis according to clinical history, viral etiology, and levels of acute severity.

Nonetheless, our findings may help identify, among children hospitalized for bronchiolitis, subgroups with particularly elevated risk of developing recurrent wheezing and asthma.

In summary, our findings support the existence of several entities under the clinical diagnosis of bronchiolitis, differentially associated with long-term outcomes such as development of recurrent wheezing. Moreover, the associations between the severe bronchiolitis profiles and several objectively measured biomarkers suggest a biological basis for the observed heterogeneity. Further work aiming at phenotyping and endotyping bronchiolitis through clinical, epidemiological and molecular approaches (including –“omics” data, e.g., host genome, transcriptome, epigenome) ¹⁰ should help improve disease management and clarify the relation of infant bronchiolitis with the development of asthma.

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Table 1. Description of the infants hospitalized for severe bronchiolitis (baseline), according to the profiles (A to C) identified by latent class analysis

	All	Profiles		
		A	B	C
n (%)	921	136 (15%)	452 (49%)	333 (36%)
History of breathing problems, %	20	44	12	23
History of eczema, %	15	23	14	13
Wheeze at ED presentation, %	63	69	56	69
Cough at ED presentation, %	96	92	100	94
Retractions at ED presentation, %				
None	19	25	22	12
Mild	44	45	55	28
Moderate to severe	37	30	23	60
Hospital length-of-stay (days), %				
< 3	60	89	85	14
3-6	32	10	15	65
≥7	8	1	0	21
Highest respiratory rate during entire hospital stay ≥60 (breath per min), %	50	22	27	94
Viral etiology				
RSV, %	82	5	99	83
Rhinovirus, %	20	54	13	17

Results presented as % (observed proportion in the study population and within profiles A to C). ED, emergency department; RSV, respiratory syncytial virus. Latent class analysis was performed based on the nine variables presented in the table, selected from a larger set of variables including many clinical factors (main features typically monitored in clinical practice, including data from ED presentation to inpatient discharge).

Table 2. Characteristics of the children according to severe bronchiolitis profiles

	All	Profiles			P*
		A	B	C	
n (%)	921	136 (15%)	452 (49%)	333 (36%)	
Socio-demographic characteristics					
Age (months), %					
<2	30	21	28	35	<0.001
2-5.9	46	37	49	45	
6-11.9	25	43	23	20	
Girls, %	40	37	41	39	0.64
Race/ethnicity, %					
Non-Hispanic white	45	35	47	46	0.05
Non-Hispanic black	23	25	23	21	
Hispanic	30	38	28	29	
Other	2	1	2	4	
Clinical history					
Parental history of asthma, %	33	38	32	33	0.36
History of antibiotic use, %	32	40	32	29	0.04
History of corticosteroid use, %	15	26	13	14	<0.001
Characteristics of acute bronchiolitis					
ED presentation					
Fever, %	26	22	28	24	0.37
Inadequate oral intake, %	61	48	59	68	<0.001
ICU and intubation/CPAP, %					
No ICU	85	96	96	65	<0.001
ICU without intubation/CPAP	10	2	4	21	
ICU with intubation/CPAP	5	2	0	14	
Viral etiology, %					
RSV only	59	0	74	62	<0.001
Rhinovirus only	6	32	0	3	
RSV and rhinovirus	12	5	13	14	
RSV and other non-rhinovirus	11	0	12	13	
Rhinovirus and other non-RSV	3	16	0	1	
Non-RSV and non-rhinovirus	10	46	1	8	
Number of pathogens, %					
No pathogen identified	3	15	1	2	<0.001
1	70	57	74	69	
2	22	24	22	22	
≥3	4	4	3	6	

ED, emergency department; RSV, respiratory syncytial virus. Results in bold are statistically significant. * Chi-square tests.

Table 3. Association of severe bronchiolitis profiles with atopy/allergy markers, vitamin D status, and serum cathelicidin

	All	Profile A	Profile B	Profile C
Food or aero-allergen sensitization*				
%	20	18	20	21
Adjusted† OR (95% CI)	.	0.68 (0.41-1.15)	1 (<i>reference</i>)	1.10 (0.76-1.57)
Total IgE, ku/L				
GM (IQR)	5.8 (1.9-12.2)	8.7 (1.9-20.6)	5.4 (1.9-10.4)	5.4 (1.9-12.2)
>100, %	3	8	2	2
Adjusted† OR (95% CI)	.	2.13 (0.79-5.70)	1 (<i>reference</i>)	0.84 (0.27-2.63)
Blood eosinophil count‡, cells/μL				
Median (IQR)	114 (16 – 238)	167 (29-370)	123 (20-218)	152 (0-216)
>300, %	18	33	15	16
Adjusted† OR (95% CI)	.	3.75 (2.03-6.93)	1 (<i>reference</i>)	1.03 (0.61-1.74)
Serum 25(OH)D, ng/mL				
Median (IQR)	26 (18-33)	26 (19-35)	27 (19-33)	26 (17-32)
≥30, %	37	40	38	34
Adjusted† OR (95% CI)	.	1.00 (0.66-1.51)	1 (<i>reference</i>)	0.96 (0.70-1.31)
Serum LL-37, ng/mL				
Median (IQR)	46 (34-62)	54 (40-71)	46 (34-58)	46 (34-60)
>46, %	49	62	47	46
Adjusted† OR (95% CI)	.	1.84 (1.23-2.75)	1 (<i>reference</i>)	1.10 (0.82-1.47)

* Defined as having ≥ 1 positive value for allergen-specific IgE (171 infants had ≥ 1 positive value for food allergens, and 15 infants had ≥ 1 positive value for aero-allergens). † Adjusted for age, sex and race/ethnicity. ‡ Highest eosinophil count during inpatient stay, available for 551 infants (60%); rate of missing value did not differ according to profiles ($P=0.81$). Results in bold are statistically significant. OR, odds ratio; CI, confidence interval; IQR, interquartile range; GM, geometric mean; IgE, Immunoglobulin E.

Table 4. Association of severe bronchiolitis profiles with nasopharyngeal microbiota profiles

Nasopharyngeal microbiota profile (n=912)	Severe bronchiolitis profiles						
	All, %	Profile A		Profile B (<i>reference</i>)		Profile C	
		%	OR [†] (95% CI)	%	OR [†] (95% CI)	%	OR [†] (95% CI)
<i>Moraxella</i> -dominant	22	29	1.44 (0.84-2.46)*	23	1	17	0.75 (0.49-1.14)
<i>Haemophilus</i> -dominant	19	29	2.31 (1.31-4.06)	13	1	24	2.04 (1.32-3.16)
Mixed profile (<i>reference</i>)	31	27	1	32	1	31	1
<i>Streptococcus</i> -dominant	28	16	0.61 (0.34-1.11)	32	1	29	0.93 (0.64-1.35)

[†]Multinomial logistic regression model adjusted for age, sex, race/ethnicity, lifetime history of antibiotic use, history of corticosteroid use, and use of antibiotics during the ED visit. Results in bold are statistically significant for comparison of profile A or profile C *vs.* profile B. *Statistically significant difference for comparison of profile A *vs.* C. OR, odds ratio; CI, confidence interval. For both microbiota profiles and severe bronchiolitis profiles, the largest group was used as reference category in adjusted analyses.

Table 5. Associations between severe bronchiolitis profile at baseline and respiratory outcomes at age 3 years

	n	Recurrent wheezing†		ICS or montelukast use‡		Doctor-diagnosed asthma§	
		n event	HR (95% CI)	n event	HR (95% CI)	n event	OR (95% CI)
Profile A	136	59	2.64 (1.90-3.68)*	64	2.55 (1.85-3.51)*	49	2.79 (1.78-4.39)*
Profile B	452	97	1 (<i>reference</i>)	109	1 (<i>reference</i>)	78	1 (<i>reference</i>)
Profile C	333	95	1.51 (1.14-2.01)	123	1.84 (1.42-2.40)	72	1.38 (0.95-2.00)

Cox proportional hazard models (recurrent wheezing, ICS or montelukast use) or multinomial logistic regression model (doctor-diagnosed asthma), adjusted for age at baseline, sex and race/ethnicity. † Having at least 2 corticosteroid-requiring exacerbations in 6 months, or having at least 4 wheezing episodes in one year that last at least one day and affect sleep. ‡Parent-reported use of ICS or montelukast. § Parental report of doctor-diagnosed asthma, available for 837 children (91%). Results in bold are statistically significant for comparison of profile A or profile C vs. profile B. *Statistically significant difference for comparison of profile A vs. C. ICS, inhaled corticosteroids; HR, hazard ratio; OR, odds ratio; CI, confidence interval.

FIGURE LEGENDS

Figure 1. Graphical summary of the severe bronchiolitis profiles identified by latent class analysis. Profiles are displayed according to their main distinctive characteristics (bronchiolitis severity, viral etiology, history of breathing problems / eczema).

Overlaps between the profiles are proportional to class membership probabilities (e.g., infants assigned to profile B had 5% probability to belong to profile A and 12% probability to belong to profile C).

Figure 2. Kaplan-Meier survival curve for the risk of developing recurrent wheezing by age 3 years, according to severe bronchiolitis profile at baseline (severe bronchiolitis hospitalization). Time in days since hospital discharge; “Survival” indicates absence of recurrent wheezing. Log-rank test: $p < 0.001$.



