Supplementary Material

A clustering approach to identify severe bronchiolitis profiles in children

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Methods

Nasopharyngeal aspirate collection and testing

Nasopharyngeal aspirate collection was performed using a standardized protocol in both the U.S. and Finnish study.[1,2] Designated site personnel were trained using a lecture, written instructions, and video. All of the sites used the same collection equipment (Medline Industries) and collected the samples within 24 hours of a child's arrival to the medical unit or ICU. Once collected, the nasopharyngeal aspirate sample was added to transport medium, immediately placed on ice, and then stored at −80°C, before analysis at Baylor College of Medicine.

Polymerase chain reaction assay

All polymerase chain reaction (PCR) assays were conducted as singleplex or duplex 2-step real-time PCR. Real-time reverse transcriptase– PCR was used for the detection of RNA respiratory viruses, which included RSV types A and B; RV; parainfluenza virus types 1, 2, and 3; influenza virus types A and B; human metapneumovirus; coronaviruses NL-65, HKU1, OC43, and 229E; and enterovirus. Real-time PCR was used for the detection of DNA pathogens, which included adenovirus, *Mycoplasma pneumoniae*, and *Bordetella pertussis*. These tests are routinely conducted in the central laboratory of one of the investigators (P.A.P.), and details of the primers and probes have been described.[3-5]

Statistical analyses

Multiple Correspondence Analysis (MCA)

The purpose of the MCA was to reduce the number of variables to introduce in the LCA model, in particular in order to avoid selecting several variables representing similar dimensions. Thus, we selected only variables with the highest contributions to the first axes.

MCA in MARC-30 U.S.

A set of 18 variables were included in the MCA: (a) medical history (history of wheezing, history of eczema, parental history of asthma); (b) clinical characteristics at time of ED presentation: duration of symptoms before presentation to the hospital, fever, respiratory rate, retraction severity, oxygen saturation, respiratory symptoms (cough, wheeze, apnea), and inadequate oral intake; (c) characteristics of the hospital course: length-of-stay, intensive care unit (ICU) admission with or without mechanical ventilation (i.e., intubation or continuous positive airway pressure [CPAP]), highest respiratory rate, most severe retractions during the inpatient stay; and (c) viral etiology, with a focus on respiratory syncytial virus (RSV) and rhinovirus.

Results of the MCA are presented in Table S1. Dimensions 1, 2 and 3 accounted for 10%, 7% and 6% of the variance respectively. The first dimension was mostly characterized by indicators of the severity of the disease (ICU admission and intubation or CPAP, length-of-stay, severity of retractions, respiratory rate). The second dimension was characterized by wheezing and cough at ED presentation, history of eczema and some severity indicators (apnea, retractions). The third dimension was characterized by history of wheezing and by the type of virus infection (especially non-RSV).

In order to limit the number of parameters in the LCA model, only variables contributing the most to each dimension were selected for the LCA. In particular, among all the variables

indicating a more severe clinical course (ICU and intubation or CPAP, length-of-stay, higher respiratory rate), only the length-of-stay was included in the LCA. A similar classification was observed when length-of-stay was replaced by ICU and intubation or CPAP in the LCA (sensitivity analysis, not shown).

Sensitivity analyses among children younger than 12 months (infants, n=1,816), are presented in Tables S3 and S4.

MCA in MARC-30 Finland

The MCA was conducted as closely as possible to MARC-30 U.S. analysis. A set of 13 variables were included in the MCA. Because the prevalence of apnea at ED presentation (2%) and ICU admission (4%) were very low in the Finnish cohort, these two variables were not included in the MCA. Similarly, because length-of-stay was shorter than in the U.S. population, it was studied in 2 categories only (<3 vs. ≥3 days) in MARC-30 Finland. Finally, there was a higher rate of missing values (>15%) for respiratory rate (ED presentation and inpatient stay) and retractions during the inpatient stay. These variables either had low contributions in MARC-30 U.S. MCA, or contributed to dimensions (disease severity) which could be represented by other variables. Thus, they were not included in MARC-30 Finland MCA.

Results of the MCA are presented in Table S2. Dimensions 1, 2 and 3 accounted for 16%, 10% and 8% of the variance respectively. The first dimension was mostly characterized by history of wheezing, inadequate oral intake and viral etiology. The second dimension was characterized by ED presentation characteristics: wheezing, cough and retractions. The third dimension was characterized by history of wheezing and eczema, and length-of-stay.

Latent Class Analysis

All subjects were included in the LCA models since missing data for the variables are handled in the procedure. A high proportion of observations (MARC-30 U.S.: 88%; MARC-30 Finland: 86%) were "fully observed" (i.e., did not have any missing value for the variables entered in the LCA model). For each given number of classes, the model was estimated 10 times, and the model with the highest likelihood was selected.

To select the number of class, models with 2 to 6 classes were examined with regard to the Bayesian Information Criteria (BIC, Supplementary Figure 1), and we retained the solution with the lowest BIC. The mean class membership probability range was 74%-85% in MARC-30 U.S. (4-class model) and 85%-92% in MARC-30 Finland (3-class model).

Statistical software

Analyses were performed with R version 3.0.2 (The R Project for Statistical Computing, www.r-project.org). The R poLCA package (a package for polytomous variable latent class analysis) was used for the LCA model.[6]

References

1 Mansbach JM, Piedra PA, Teach SJ, *et al.* Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med* 2012;**166**:700–6.

2 Jartti T, Aakula M, Mansbach JM, *et al.* Hospital length-of-stay is associated with rhinovirus etiology of bronchiolitis. *Pediatr Infect Dis J* 2014;**33**:829–34.

3. Beckham JD, Cadena A, Lin J, et al. Respiratory viral infections in patients with chronic, obstructive pulmonary disease. J Infect. 2005;**50**:322–330.

4. Knorr L, Fox JD, Tilley PA, et al. Evaluation of real-time PCR for diagnosis of Bordetella pertussis infection. BMC Infect Dis. 2006;**6**:62.

5. Winchell JM, Thurman KA, Mitchell SL, et al. Evaluation of three real-time PCR assays for detection of Mycoplasma pneumoniae in an outbreak investigation. J Clin Microbiol. 2008;**46**:3116–3118.

6. Linzer DA, Lewis JB. poLCA: An R Package for Polytomous Variable Latent Class Analysis. *J Stat Softw* 2011;**42**:1–29.

Table S1. Contribution of the variables to the 3 first dimensions in the multiple correspondence analysis, MARC-30 U.S. (n=2,207)

	Dim 1	Dim 2	Dim 3
Medical history			
History of wheezing: No	0.05	1.91	1.55
History of wheezing: Yes	0.17	6.64	5.38
History of eczema: No	0.05	1.19	0.31
History of eczema: Yes	0.32	7.24	1.86
Parental history of asthma: No	0.03	0.74	0.12
Parental history of asthma: Yes	0.06	1.62	0.27
ED presentation			
Difficulty breathing: None	0.13	2.33	0.15
Difficulty breathing began <1 day	0.02	0.07	3.74
Difficulty breathing began ≥1 day	0.00	0.01	1.64
Fever: No	0.19	0.04	0.10
Fever: Yes	0.46	0.10	0.24
Respiratory rate <60	0.79	1.27	0.23
Respiratory rate ≥60	2.03	3.29	0.59
Retractions: None	1.95	13.00	0.90
Retractions: Mild	0.78	1.10	0.95
Retractions: Moderate to severe	5.05	3.35	0.12
O2 sat by pulse or ABG: <90	3.35	0.04	3.29
O2 sat by pulse or ABG: 90-93.9	0.08	0.16	1.12
O2 sat by pulse or ABG: ≥ 94	0.73	0.08	0.04
Cough: No	0.28	4.34	0.66
Cough: Yes	0.04	0.66	0.10
Wheeze: No	0.08	16.48	0.02
Wheeze: Yes	0.05	9.02	0.01
Apnea: No	0.16	0.37	0.25
Apnea: Yes	2.22	5.22	3.50
Inadequate oral intake: missing	0.12	0.21	0.21
Inadequate oral intake: No	2.93	0.05	0.10
Inadequate oral intake: Yes	2.46	0.00	0.32
Hospitalization course			
No ICU	2.91	0.08	0.56
ICU without intubation or CPAP	4.68	0.18	0.06
ICU with intubation or CPAP	10.40	2.27	5.17
Length-of-stay <3 days	8.00	0.04	0.91
Length-of-stay 3-6 days	3.10	1.33	6.26
Length-of-stay ≥ 7 days	10.97	2.79	5.80
Highest respiratory rate <60	6.47	0.01	0.22
Highest respiratory rate ≥60	10.23	0.01	0.35
Retractions: None	6.56	4.29	1.03
Retractions: Mild	0.00	2.93	1.17
Retractions: Moderate to severe	10.84	0.02	0.09

Virus			
RSV: No	0.88	0.81	21.43
RSV: Yes	0.33	0.31	8.15
Rhinovirus: No	0.01	1.12	5.37
Rhinovirus: Yes	0.03	3.27	15.65

CPAP - Continuous Positive Airway Pressure; ICU: Intensive Care Unit; RSV: Respiratory Syncytial Virus.
Variables in bold are the 9 variables selected to be included in the Latent Class Analysis.

Table S2. Contribution of the variables to the 3 first dimensions in the multiple correspondence analysis, MARC30-Finland (n=408)

	Dim 1	Dim 2	Dim 3
Medical history			
History of wheezing: No	4.76	0.30	3.86
History of wheezing: Yes	9.43	0.59	7.66
History of eczema: No	0.98	0.01	9.89
History of eczema: Yes	2.69	0.04	27.2
Parental history of asthma: No	0.01	0.91	0.40
Parental history of asthma: Yes	0.04	2.78	1.22
ED presentation			
Difficulty breathing: None or began <1 day	3.36	0.09	5.50
Difficulty breathing: Began ≥1 day	1.41	0.04	2.32
Fever: No	0.17	0.02	3.64
Fever: Yes	0.40	0.04	8.53
Retractions: None	1.31	16.2	0.38
Retractions: Mild	2.11	0.02	5.44
Retractions: Moderate to severe	4.39	5.15	3.60
O2 sat by pulse or ABG: <90	4.72	1.83	1.31
O2 sat by pulse or ABG: 90-93.9	0.81	0.69	3.09
O2 sat by pulse or ABG: ≥ 94	1.29	0.00	0.32
Cough: No	0.69	22.5	0.27
Cough: Yes	0.35	11.3	0.14
Wheeze: No	4.37	15.9	0.37
Wheeze: Yes	1.92	6.99	0.16
Inadequate oral intake: No	1.37	1.08	0.09
Inadequate oral intake: Yes	5.88	4.63	0.38
Hospitalization course			
Length-of-stay <3 days	0.98	0.34	3.94
Length-of-stay ≥ 3 days	2.22	0.77	8.95
Virus			
RSV: No	10.2	2.57	0.46
RSV: Yes	12.8	3.22	0.58
Rhinovirus: No	6.54	0.59	0.08
Rhinovirus: Yes	14.9	1.33	0.19

CPAP - Continuous Positive Airway Pressure; ICU: Intensive Care Unit; RSV: Respiratory Syncytial Virus.
Variables in bold are the 7 variables selected to be included in the Latent Class Analysis.

Table S3. Contribution of the variables to the 3 first dimensions in the multiple correspondence analysis, MARC-30 U.S. infants (aged < 12 months, n=1,896)

	Dim 1	Dim 2	Dim 3
Medical history			
History of wheezing: No	0.01	0.84	2.28
History of wheezing: Yes	0.03	3.57	9.71
History of eczema: No	0.04	0.96	0.66
History of eczema: Yes	0.25	6.70	4.60
Parental history of asthma: No	0.01	0.86	0.48
Parental history of asthma: Yes	0.02	1.80	1.01
ED presentation			
Difficulty breathing: None	0.19	2.44	0.00
Difficulty breathing began <1 day	0.06	0.06	2.91
Difficulty breathing began ≥1 day	0.01	0.19	1.11
Fever: No	0.14	0.12	0.13
Fever: Yes	0.35	0.30	0.34
Respiratory rate <60	0.79	1.79	0.03
Respiratory rate ≥60	1.89	4.29	0.06
Retractions: None	2.15	12.1	0.10
Retractions: Mild	0.73	1.22	0.42
Retractions: Moderate to severe	5.24	2.72	0.25
O2 sat by pulse or ABG: <90	3.66	0.40	1.84
O2 sat by pulse or ABG: 90-93.9	0.30	0.54	2.21
O2 sat by pulse or ABG: ≥ 94	0.94	0.01	0.03
Cough: No	0.22	4.60	0.20
Cough: Yes	0.04	0.77	0.03
Wheeze: No	0.01	14.3	0.50
Wheeze: Yes	0.00	8.69	0.31
Apnea: No	0.22	0.59	0.10
Apnea: Yes	2.86	7.61	1.32
Inadequate oral intake: missing	0.05	0.40	0.37
Inadequate oral intake: No	2.91	0.03	0.39
Inadequate oral intake: Yes	2.65	0.02	0.89
Hospitalization course			
No ICU	2.89	0.33	0.38
ICU without intubation or CPAP	3.87	0.02	0.17
ICU with intubation or CPAP	11.1	4.28	2.41
Length-of-stay <3 days	8.15	0.10	0.75
Length-of-stay 3-6 days	2.84	2.63	4.22
Length-of-stay ≥ 7 days	11.4	4.92	3.14
Highest respiratory rate <60	6.88	0.08	0.19
Highest respiratory rate ≥60	9.54	0.12	0.27

Table S3 (continued). Contribution of the variables to the 3 first dimensions in the multiple correspondence analysis, MARC-30 U.S. (n=1,896 infants)

	Dim 1	Dim 2	Dim 3
Retractions: None	6.61	4.96	0.25
Retractions: Mild	0.00	3.33	0.39
Retractions: Moderate to severe	10.1	0.00	0.09
Virus			
RSV: No	0.56	0.08	23.0
RSV: Yes	0.19	0.03	7.75
Rhinovirus: No	0.01	0.32	6.18
Rhinovirus: Yes	0.02	0.94	18.4

CPAP - Continuous Positive Airway Pressure; ICU: Intensive Care Unit; RSV: Respiratory Syncytial Virus.
Variables in bold are the 9 variables selected to be included in the Latent Class Analysis.

Table S4. Description of the infants (aged < 12 months) hospitalized for severe bronchiolitis according to the profiles (A to D) identified by Latent Class Analysis (LCA), for the clinical characteristics introduced in the LCA model, MARC-30 U.S., n=1,896 infants

	All	Profiles			
		A (10%)	B (34%)	C (39%)	D (17%)
History of wheezing	19	48	17	16	11
History of eczema	12	32	14	8	4
Wheeze at ED presentation	62	87	80	61	8
Cough at ED presentation	85	90	94	82	72
Retractions at ED presentation					
None	24	18	21	12	60
Mild	45	46	56	40	36
Moderate to severe	31	36	23	48	4
Hospital length-of-stay					
< 3 days	54	75	78	16	77
3-6 days	34	21	21	57	18
≥7 days	12	4	<1	27	5
Retractions during hospital stay					
None	32	36	49	2	68
Mild	47	52	51	47	32
Moderate to severe	21	12	<1	51	<1
Viral etiology					
RSV	74	24	87	84	62
Rhinovirus	25	66	13	24	24

Results are expressed as % (observed proportion in the study population ["All"], and probability of individuals presenting the characteristics within profiles A to D).

Abbreviations: ED, emergency department; RSV, respiratory syncytial virus.

Profile A: history of wheezing/eczema, wheezing at ED presentation, more often rhinovirus. Profile B: wheezing at ED presentation, more often RSV only. Profile C: most severe. Profile D: non-wheezing at ED presentation, least severe.

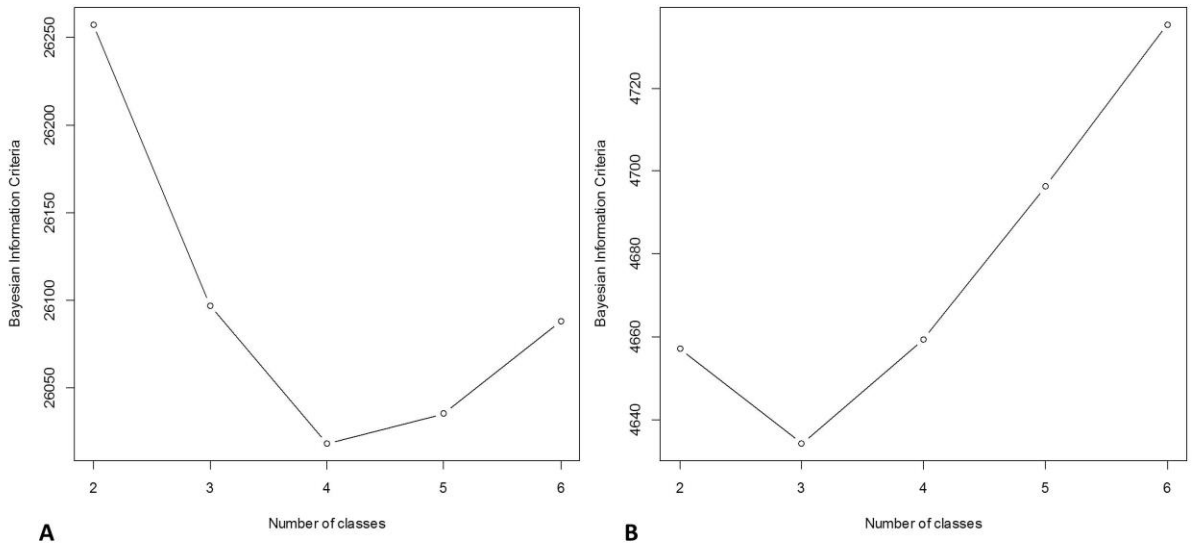

Figure S1. Model fit information for 2- to 6-class LCA models, in [A] MARC-30 U.S. (n=2,207) and [B] MARC30-Finland (n=408).

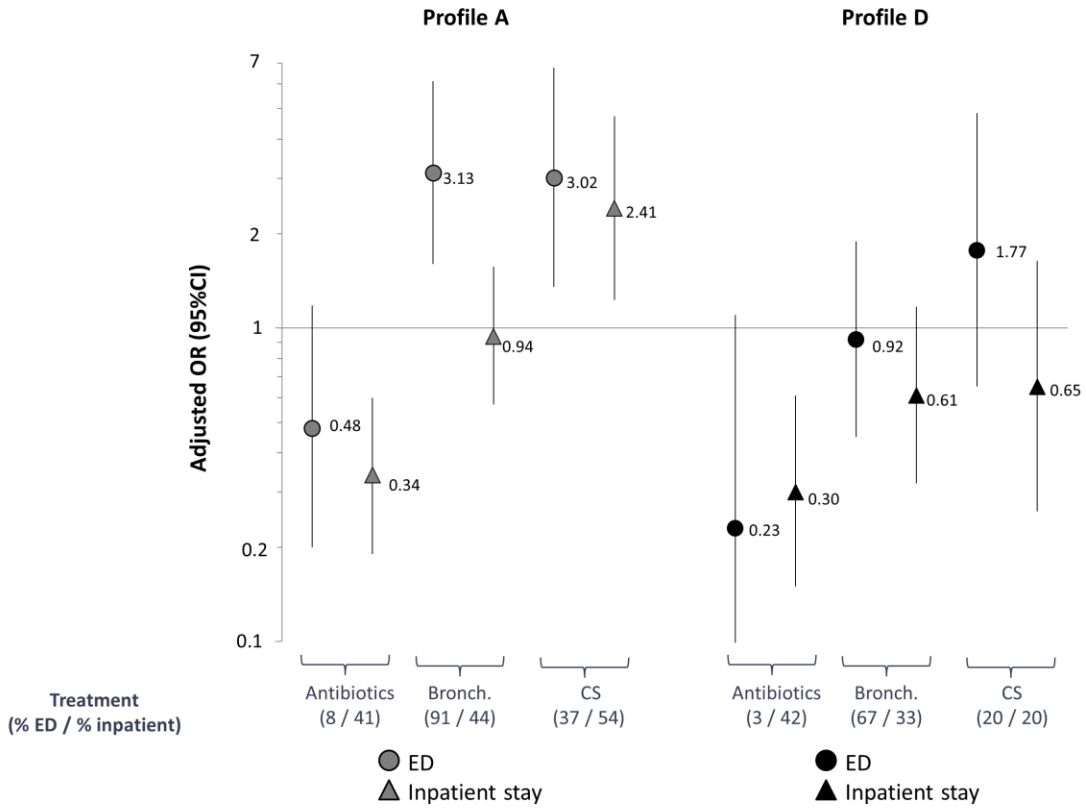

Figure S2. Associations between profiles (A, BC and D) identified by Latent Class Analysis (LCA) and treatment (antibiotics, inhaled bronchodilators [bronch.], and systemic or inhaled corticosteroids [CS]) at emergency department (ED) presentation and during inpatient stay, in MARC-30 Finland, n=408 children younger than 2 years old

Results are presented as percentage of patients receiving treatment at ED presentation (% ED) and during inpatient stay (%inpatient), and odds ratio (OR) and 95% confidence intervals (CI), adjusted for age, and sex. Profile BC was used as the reference category (% ED / % inpatient for antibiotics: 9 / 59; bronchodilators: 56 / 44; corticosteroids: 6 / 14).

Profile A: history of wheezing/eczema, wheezing at ED presentation, more often rhinovirus. Profile BC: wheezing at ED presentation, more often RSV only, most severe. Profile D: non-wheezing at ED presentation, least severe