

# Prospective Multicenter Study of Viral Etiology and Hospital Length of Stay in Children With Severe Bronchiolitis

Jonathan M. Mansbach, MD; Pedro A. Piedra, MD; Stephen J. Teach, MD, MPH; Ashley F. Sullivan, MS, MPH; Tate Forgey, MS; Sunday Clark, MPH, ScD; Janice A. Espinola, MPH; Carlos A. Camargo Jr, MD, DrPH; for the MARC-30 Investigators

**Objective:** To determine whether hospital length of stay (LOS) for acute bronchiolitis is influenced by the infecting pathogen.

**Design:** A prospective observational cohort study was performed during 3 consecutive years.

**Setting:** Sixteen US hospitals participated in the study.

**Participants:** Children younger than 2 years hospitalized with bronchiolitis were included.

**Main Exposure:** The results of nasopharyngeal aspirate polymerase chain reaction pathogen testing served as the main exposure.

**Main Outcome Measure:** Hospital LOS was determined.

**Results:** Of 2207 participants, 72.0% had respiratory syncytial virus (RSV) and 25.6% had human rhinovirus (HRV); the incidence of each of the other viruses and bacteria was 7.8% or less. Multiple pathogen infections were present in 29.8% of the children. There were 1866 children (84.5%) with RSV and/or HRV. Among these 1866

children, the median age was 4 months and 59.5% were male. The median LOS was 2 days (interquartile range, 1-4 days). Compared with children who had only RSV, an LOS of 3 or more days was less likely among children with HRV alone (adjusted odds ratio [AOR], 0.36; 95% CI, 0.20-0.63;  $P < .001$ ) and those with HRV plus non-RSV pathogens (AOR, 0.39; 95% CI, 0.23-0.66;  $P < .001$ ) but more likely among children with RSV plus HRV (AOR, 1.33; 95% CI, 1.02-1.73;  $P = .04$ ), controlling for 15 demographic and clinical factors.

**Conclusions:** In this multicenter study of children hospitalized with bronchiolitis, RSV was the most common virus detected, but HRV was detected in one-quarter of the children. Since 1 in 3 children had multiple virus infections and HRV was associated with LOS, these data challenge the effectiveness of current RSV-based cohorting practices, the sporadic testing for HRV in bronchiolitis research, and current thinking that the infectious etiology of severe bronchiolitis does not affect short-term outcomes.

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**B**RONCHIOLITIS IS ONE OF THE most common infectious respiratory conditions of early childhood<sup>1</sup> and the leading cause of hospitalization for infants.<sup>1,2</sup> The most common pathogen associated with severe bronchiolitis (ie, requiring hospitalization) is respiratory syncytial virus (RSV)<sup>3</sup> and the second most common is human rhinovirus (HRV).<sup>4,5</sup> With the advent of molecular amplification techniques, however, it has become clear that a diverse group of pathogens is associated with severe bronchiolitis and that these pathogens may infect children in isolation or in combination as coinfections.<sup>5-7</sup>

The clinical relevance of identifying the specific pathogen or combination of pathogens infecting a child with severe bronchiolitis remains unclear.<sup>8-10</sup> As a result, children

with bronchiolitis, no matter the infecting pathogen, are considered to have essentially the same disease. Indeed, the 2006 American Academy of Pediatrics bronchiolitis clinical practice guideline<sup>8</sup> recommends that clinicians limit viral diagnostic testing when

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caring for children with bronchiolitis. Children with HRV, however, may have different short-term and long-term outcomes than children with RSV.<sup>5,11-14</sup> Specifically, children with HRV bronchiolitis may have shorter acute clinical courses<sup>5,11</sup> and may be at increased risk of recurrent wheezing and asthma<sup>12-14</sup> compared with children with RSV bronchiolitis. To examine the clinical use-

Author Affiliations are listed at the end of this article.

Group Information: The MARC-30 (30th Multicenter Airway Research Collaboration) investigators are listed at the end of this article.

fulness of identifying infectious etiology, we conducted a prospective, multicenter, multiyear study of more than 2000 children hospitalized with bronchiolitis. We hypothesized that children infected with HRV alone would have a shorter hospital length of stay (LOS) than would children infected with RSV alone.

## METHODS

### STUDY DESIGN

We conducted a prospective, multicenter cohort study for 3 consecutive years during the 2007–2010 winter seasons as part of the Multicenter Airway Research Collaboration, a program of the Emergency Medicine Network (<http://www.emnet-usa.org>). The number of participating sites varied during the 3 years: 13 sites in year 1, 16 sites in year 2, and 14 sites in year 3. Each month from November 1 until March 31, site investigators across 12 states used a standardized protocol to enroll a target number of consecutive patients from the inpatient units and the intensive care unit (ICU). Once the site reached its target enrollment for that month, the investigators would stop enrollment until the beginning of the next month.

All patients were treated at the discretion of the treating physician. Inclusion criteria were an attending physician's diagnosis of bronchiolitis, age younger than 2 years, and the ability of the parent/guardian to give informed consent. The exclusion criterion was previous enrollment. All consent and data forms were translated into Spanish. The institutional review board at each of the 16 participating hospitals approved the study.

### DATA COLLECTION

The investigators conducted a structured interview that assessed patients' demographic characteristics, medical and environmental history, duration of symptoms, and details of the acute illness. Relevant comorbid medical disorders included respiratory, cardiac, neurologic, gastrointestinal, and immunologic diseases. Emergency department and daily hospital medical records provided further clinical data, including respiratory rates, daily respiratory rate trends, clinical assessment of degree of retractions (collapsed for analysis into none, mild, and moderate/severe), oxygen saturation, daily oxygen saturation trends, medical management, and disposition. These data were reviewed at the Emergency Medicine Network Coordinating Center, and site investigators were queried about missing data and discrepancies identified by manual data checks.

### NASOPHARYNGEAL ASPIRATE COLLECTION AND TESTING

Nasopharyngeal aspirate collection was performed using a standardized protocol. 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  $-80^{\circ}\text{C}$ . Frozen samples were batch shipped on dry ice overnight to the central laboratory at Baylor College of Medicine, where they continued to be stored at  $-80^{\circ}\text{C}$ .

### 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 vi-

ruses, which included RSV types A and B; HRV; parainfluenza virus types 1, 2, and 3; influenza virus types A and B; the 2009 novel influenza A virus (H1N1); 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.<sup>15–17</sup>

### STATISTICAL ANALYSIS

All analyses were performed using commercial software (Stata 11.2; StataCorp). Data are presented as proportions with 95% CIs and medians with interquartile ranges. Our primary analyses focused on RSV and HRV, the most commonly detected viruses in children with severe bronchiolitis. For the purposes of this analysis, we combined RSV-A with RSV-B because the clinical distinction between these subtypes of RSV was unremarkable. For analyses, we created a categorical variable that reflected the possible combinations of RSV/HRV status: (1) RSV only, (2) HRV only, (3) RSV in combination with HRV, (4) RSV in combination with non-HRV pathogens, and (5) HRV in combination with non-RSV pathogens.

We performed univariate analyses using the  $\chi^2$  test, Fisher exact test, and Kruskal-Wallis test, as appropriate. All *P* values were 2-tailed, with *P* < .05 considered statistically significant. Multivariable logistic regression analyses were conducted to evaluate independent predictors of a longer LOS ( $\geq 3$  days; defined using the median value of 2 days) and other measures of severity: ICU admission and continuous positive airway pressure (CPAP)/intubation. Factors were selected for inclusion in the model if they were found to be associated with the outcome in unadjusted analyses (*P* < .20) or were potentially clinically significant. All regression models account for potential clustering by site. To further investigate independent predictors of LOS, a zero-truncated negative binomial model was also used to evaluate the relationship between demographic and clinical factors and LOS in days (continuous outcome). Children who were hospitalized for less than 1 day were assigned 0.5 days' LOS. Results of the zero-truncated negative binomial model are reported as incidence rate ratios (IRRs) with 95% CIs.

## RESULTS

Of 3910 eligible children with severe bronchiolitis, 2207 children (56.4%) were enrolled. Enrolled and nonenrolled children were similar in age and sex (*P* > .05), but enrolled children were more likely to be white (61.4% vs 49.9%; *P* < .001) and Hispanic (36.3% vs 28.9%; *P* < .001). Enrolled children also were less likely to have an LOS of 3 or more days (44.4% vs 48.7%; *P* = .007).

Of the 2207 enrolled children, 1410 children (63.9%) had a single virus infection and 658 (29.8%) had 2 or more viruses; the remaining 139 children (6.3%) had no pathogen identified from our testing panel. Among all enrolled children, the pathogens detected were RSV-A (42.6%) and RSV-B (30.1%); HRV (25.6%); parainfluenza virus types 1, 2, and 3 (2.6%); influenza A, B, and the 2009 novel H1N1 (1.2%); human metapneumovirus (7.0%); coronaviruses NL-65, HKU1, OC43, and 229E (7.1%); enterovirus (4.9%); adenovirus (7.8%); *M pneumoniae* (0.9%); and *B pertussis* (0.2%). Children with RSV and/or HRV represented 1866 (84.5%) of the 2207 children. At least 1 other virus was detected in 32.3% of chil-

**Table 1. Demographic Characteristics, Medical History, and Clinical Course of Children With Severe Bronchiolitis by RSV and HRV Infection Status**

Characteristic	%					P Value
	RSV Only (n = 1075)	HRV Only (n = 167)	RSV Plus HRV (n = 287)	RSV Plus Non-HRV (n = 227)	HRV Plus Non-RSV (n = 110)	
Age, mo						
<1.0	18.6	6.0	8.0	5.3	1.8	<.001
1.0-1.9	19.3	13.2	18.1	11.0	7.3	
2.0-3.9	22.9	18.0	26.1	17.6	13.6	
4.0-5.9	12.1	10.2	18.5	16.7	10.9	
6.0-11.9	16.2	31.1	22.0	30.0	40.9	
12.0-23.9	10.9	21.6	7.3	19.4	25.5	
Female sex	42.3	34.1	39.7	40.1	34.5	.20
Race						
White	64.0	62.9	60.3	59.9	50.0	.03
Black	21.2	26.9	27.2	27.8	32.7	
Other or missing	14.8	10.2	12.5	12.3	17.3	
Hispanic ethnicity	36.4	30.5	36.9	32.2	39.1	.39
Family history of asthma						
Neither parent	68.1	64.1	65.2	65.6	66.4	.64
Either mother or father	27.1	32.3	27.5	28.2	27.3	
Both parents	3.5	3.0	4.2	3.5	3.6	
Do not know/missing	1.3	0.6	3.1	2.6	2.7	
Maternal smoking during pregnancy	13.0	21.7	18.9	19.4	10.1	.001
Gestational age, wk						
<32	3.6	12.0	6.0	3.6	11.0	<.001
32-36	16.4	21.7	17.9	16.1	24.8	
≥37 or "full term"	80.0	66.3	76.1	80.3	64.2	
History of eczema						
No	85.7	76.0	83.3	80.6	76.4	.004
Yes	13.3	24.0	15.0	17.2	20.9	
Missing	1.0	0.0	1.7	2.2	2.7	
History of intubation	7.0	15.7	8.0	7.5	18.2	<.001
Major relevant comorbid medical disorder	14.8	31.9	17.6	25.7	31.2	<.001
Presence of apnea (medical record)	7.5	4.8	7.8	5.3	10.1	.38
Respirations/min, median (IQR)	48 (10-60)	48 (38-57)	50 (40-60)	45 (38-60)	48 (40-60)	.16
Retractions						
None	23.6	24.0	13.9	18.1	21.8	.06
Mild	42.0	36.5	42.9	42.3	38.2	
Moderate or severe	27.5	31.7	35.2	33.5	33.5	
Missing	6.8	7.8	8.0	6.2	6.4	
Oxygen saturation by pulse oximetry or ABG, %						
<90.0	10.4	13.2	11.5	9.7	16.4	.63
90.0-93.9	17.2	14.4	17.1	17.2	12.7	
≥94.0	72.4	72.5	71.4	73.1	70.9	
Oral intake						
Adequate	43.4	52.7	37.6	40.5	47.3	.01
Inadequate	43.9	32.9	48.8	42.7	33.6	
Missing	12.7	14.4	13.6	16.7	19.1	
ICU admission	17.2	15.8	18.1	17.1	17.6	.98
Intubation and/or CPAP during admission	7.5	5.5	10.0	5.4	5.6	.23
Length of stay ≥3 d	47.6	27.5	53.7	46.7	27.3	<.001

Abbreviations: ABG, arterial blood gas; CPAP, continuous positive airway pressure; HRV, human rhinovirus; ICU, intensive care unit; IQR, interquartile range; RSV, respiratory syncytial virus.

dren who tested positive for RSV, 23.3% of those who had negative test results for RSV, 70.4% of children with HRV, and 15.9% of those without HRV.

Given the high frequency of RSV and HRV, we restricted this analysis to the 1866 children with RSV and/or HRV. Among these 1866 children, the median age was 4 months (interquartile range, 2-8 months), 59.5% were male, 62.0% were white, and 35.6% were Hispanic. The median LOS was 2 days (interquartile range, 1-4 days). Moreover, 1075 children (57.6%) had RSV only, 167

(8.9%) had HRV only, 287 (15.4%) had RSV plus HRV, 227 (12.2%) had RSV plus non-HRV pathogens, and 110 (5.9%) had HRV plus non-RSV pathogens. We examined the demographic and clinical characteristics according to these 5 groups (**Table 1**).

Unadjusted associations between various demographic and clinical characteristics and LOS (<3 days vs ≥3 days) are presented in **Table 2**. In general, younger children, white children, and those with gestational age less than 32 weeks were more likely to have a longer LOS.

**Table 2. Demographic Characteristics, Medical History, and Clinical Course of Children With Severe Bronchiolitis Associated With RSV and/or HRV by Hospital LOS and Clinical Course of Children With Severe Bronchiolitis Associated With RSV and/or HRV by Hospital LOS**

Characteristic	%		P Value
	LOS <3 d (n = 1018)	LOS ≥3 d (n = 848)	
Age, mo			
<1.0	9.4	17.8	<.001
1.0-1.9	15.0	19.1	
2.0-3.9	22.5	20.9	
4.0-5.9	13.5	13.3	
6.0-11.9	24.5	18.0	
12.0-23.9	15.1	10.8	
Female sex	38.9	42.3	
Race			
White	60.4	63.9	.01
Black	26.7	21.0	
Other or missing	12.9	15.1	
Hispanic ethnicity	34.6	36.8	.32
Family history of asthma			
Neither parent	66.1	67.8	.41
Either mother or father	28.0	27.5	
Both parents	4.2	2.8	
Do not know/missing	1.7	1.9	
Maternal smoking during pregnancy	14.3	16.5	.19
Gestational age, wk			
<32	4.1	6.5	.01
32-36	16.3	19.1	
≥37 or "full term"	79.7	74.4	
History of eczema			
No	82.1	84.7	.34
Yes	16.5	14.2	
Missing	1.4	1.2	
History of intubation	8.3	9.1	.52
Major relevant comorbid medical disorder	18.6	19.6	.57
Presence of apnea (medical record)	4.8	10.2	<.001
Respirations/min, median (IQR)	48 (40-60)	48 (40-60)	.003
Retractions			
None	25.3	16.6	<.001
Mild	43.1	39.5	
Moderate or severe	25.4	35.8	
Missing	6.1	8.0	
Oxygen saturation by pulse oximetry or ABG, %			
<90.0	6.9	16.6	<.001
90.0-93.9	16.3	17.9	
≥94.0	76.8	65.5	
Oral intake			
Adequate	49.7	35.5	<.001
Inadequate	37.2	49.8	
Missing	13.1	14.7	
ICU admission	6.0	30.4	<.001
Intubation and/or CPAP during admission	0.3	15.5	<.001
RSV/HRV status			
RSV only	55.3	60.4	<.001
HRV only	11.9	5.4	
RSV plus HRV	13.1	18.2	
RSV plus any other non-HRV pathogen	11.9	12.5	
HRV plus any other non-RSV pathogen	7.9	3.5	

Abbreviations: ABG, arterial blood gas; CPAP, continuous positive airway pressure; HRV, human rhinovirus; ICU, intensive care unit; IQR, interquartile range; LOS, length of stay; RSV, respiratory syncytial virus.

Furthermore, clinical factors, such as more severe retractions, lower oxygen saturation, apnea, inadequate oral intake, and ICU admission, were associated with a lon-

**Table 3. Multivariable Predictors of Hospital Length of Stay of 3 or More Days Among 1866 Children With Severe Bronchiolitis Associated With RSV and/or HRV<sup>a</sup>**

Characteristic	Adjusted Odds Ratio (95% CI) <sup>a</sup>	P Value
Age, mo		
<1.0	2.57 (1.73-3.82)	<.001
1.0-1.9	1.75 (1.14-2.69)	.01
2.0-3.9	1.21 (0.89-1.66)	.22
4.0-5.9	1.37 (0.94-2.00)	.11
6.0-11.9	0.99 (0.69-1.43)	.95
≥12.0	1 [Reference]	
Female sex	1.12 (0.91-1.37)	.30
Race/ethnicity		
White	1 [Reference]	
Black	0.79 (0.62-1.01)	.06
Other or missing	1.07 (0.75-1.53)	.70
Maternal smoking during pregnancy	1.15 (0.86-1.55)	.35
Gestational age, wk		
<32	2.57 (1.44-4.57)	.001
32-36	1.26 (0.94-1.68)	.12
≥37 or "full term"	1 [Reference]	
History of eczema		
No	1 [Reference]	
Yes	1.15 (0.83-1.59)	.41
Missing	0.59 (0.26-1.33)	.20
History of intubation	0.95 (0.71-1.28)	.75
Major relevant comorbid medical disorder	1.14 (0.86-1.49)	.36
Apnea (per medical record)	1.14 (0.77-1.71)	.51
Respirations/min	1.00 (0.99-1.01)	.95
Retractions		
None	1 [Reference]	
Mild	1.62 (1.23-2.12)	.001
Moderate or severe	2.05 (1.45-2.91)	<.001
Missing	1.64 (0.94-2.86)	.08
Oxygen saturation by pulse oximetry or ABG, %		
<90.0	2.06 (1.45-2.93)	<.001
90.0-93.9	1.26 (0.98-1.62)	.07
≥94.0	1 [Reference]	
Oral intake		
Adequate	1 [Reference]	
Inadequate	1.31 (0.93-1.84)	.12
Missing	1.19 (0.81-1.72)	.37
ICU	5.33 (3.01-9.44)	<.001
RSV/HRV status		
RSV only	1 [Reference]	
HRV only	0.36 (0.20-0.63)	<.001
RSV plus HRV	1.33 (1.02-1.73)	.04
RSV plus any other non-HRV pathogen	1.06 (0.67-1.69)	.79
HRV plus any other non-RSV pathogen	0.39 (0.23-0.66)	<.001

Abbreviations: ABG, arterial blood gas; HRV, human rhinovirus; ICU, intensive care unit; RSV, respiratory syncytial virus.

<sup>a</sup>Adjusted for 15 demographic and clinical characteristics as well as site.

ger LOS. Two other unadjusted multivariable models were generated with ICU and CPAP/intubation as outcomes, but RSV/HRV status did not significantly predict either severity outcome (data not shown).

The multivariable logistic regression model for an LOS of 3 or more days is reported in **Table 3**. Controlling for 15 demographic and clinical characteristics as well as site, significant independent predictors for a longer LOS were age younger than 2 months, gestational age less than 32 weeks, presence of retractions, oxygen saturation less than 90%, ICU admission, and a viral etiology. Compared with children with RSV only, those with HRV only or HRV plus any other non-RSV pathogen were less likely to have a longer LOS (both  $P < .001$ ), whereas children

with RSV plus HRV infections were more likely to have an LOS of 3 or more days ( $P=.04$ ). Even after restricting the analysis to the most common subset of children with bronchiolitis, those younger than 12 months and with a gestational age of 37 weeks or more, the results remained robust.

The zero-truncated negative binomial model, which examines LOS as a continuous outcome, showed similar results for the demographic and clinical factors presented in Table 3 with LOS as a dichotomous outcome (data not shown). This model also supported the virus findings. Compared with RSV only, the IRR for LOS was lower for children with HRV only (IRR, 0.73; 95% CI, 0.54-0.98;  $P=.04$ ) and higher for children with RSV plus HRV (IRR, 1.18; 95% CI, 1.02-1.36;  $P=.03$ ).

## COMMENT

In this large, multicenter, multiyear prospective study of children hospitalized with bronchiolitis, we found that 29.8% of the children had multiple pathogen infections. The 2 most common viral causes were RSV (72.0%) and HRV (25.6%), and children with these viruses had different short-term outcomes. In comparison with children with RSV-only infections, multivariable models demonstrated that children infected with HRV alone or in combination with non-RSV viruses had a significantly shorter LOS. Children with RSV/HRV coinfections had a significantly longer LOS, even after adjusting for clinical and demographic factors associated with severity of illness. Therefore, on the basis of this large sample from across the United States, we submit that inpatient cohorting practices may not be as effective as once believed, that researchers consider testing for HRV more routinely in bronchiolitis studies, and that clinicians and researchers reconsider conventional wisdom that the infectious etiology of severe bronchiolitis does not affect short-term outcomes.

Although several studies have found that HRV lower respiratory tract infection in early childhood is associated with later wheezing<sup>12,18,19</sup> and asthma,<sup>13,14,18</sup> the short-term outcomes are less clear. Indeed, recent studies have shown that the clinical severity of HRV bronchiolitis is less than,<sup>5</sup> no different than,<sup>4</sup> and greater than<sup>20</sup> bronchiolitis due to RSV. Specifically, Marguet and colleagues<sup>5</sup> performed a 4-center prospective study in France of 209 infants younger than 1 year with their first bronchiolitis hospitalization. In a multivariable analysis, they found that the 15 children with HRV-only infections had a reduced odds of staying in the hospital for 5 or more days (odds ratio, 0.13; 95% CI, 0.03-0.57) compared with children with RSV alone. However, a single-center study<sup>4</sup> in Spain of 318 children younger than 2 years with severe bronchiolitis found no significant difference in LOS for the 24 children with HRV alone compared with RSV alone. A single-center prospective study<sup>20</sup> in Greece of 118 children younger than 18 months with severe bronchiolitis found that the presence of HRV (ie, alone or as coinfection) increased the odds of having a clinical severity score higher than the median (adjusted odds ratio, 4.9; 95% CI, 1.2-18.7). In the present multicenter

study, we found that, on average, the 167 children with HRV-only infections had a shorter LOS than did children with RSV-only infections, even after controlling for 15 factors associated with severity of illness.<sup>21,22</sup>

It may seem intuitive that children infected with more than 1 virus should have a more severe clinical course than children infected by only 1 virus, but the data on multiple pathogen infections are unclear.<sup>23-26</sup> Interestingly, we found that children with HRV in combination with viruses other than RSV had a shorter LOS. However, when HRV was paired with RSV, children with this specific coinfection had a longer LOS than did those with RSV alone. There are few data with which to compare our results, but Marguet and colleagues<sup>5</sup> found that the 30 children with RSV/HRV coinfections had a reduced odds of staying in the hospital for 5 or more days (odds ratio, 0.26; 95% CI, 0.09-0.76) compared with children with RSV alone. A different and more acute measure of severity of illness is ICU admission or CPAP/intubation. Although Papadopoulos and colleagues<sup>20</sup> found that HRV increased an admission clinical severity score among 118 children with bronchiolitis, we did not find that the infectious etiology increased the odds of admission to the ICU or use of CPAP/intubation. Therefore, based on our data, children with RSV/HRV coinfections have a protracted severe illness but not necessarily a higher intensity of illness, as represented by the intensive care outcomes.

Although the pathophysiologic characteristics of the interactions between RSV and HRV are beyond the scope of this analysis, it is interesting that without RSV as a cofactor the clinical course of HRV parallels its more common, less severe, outpatient clinical course.<sup>18,27</sup> There are at least 2 plausible theories for the increased severity of illness of RSV/HRV coinfections. One possibility is that a diminished interferon  $\gamma$  response associated with RSV may allow for enhanced HRV replication<sup>28,29</sup>; a similar pathogenesis occurs in airway epithelial cells from people with asthma.<sup>30,31</sup> Another possibility is that RSV-infected endothelial cells increase the cell surface expression of intercellular adhesion molecule 1,<sup>32</sup> the major receptor for HRV,<sup>33,34</sup> setting the stage for a more severe HRV infection.<sup>35</sup>

Of direct relevance to all hospitals that have 2 or more beds per room are the infection control issues raised by these data. The current point-of-care virology tests used to develop care plans for children with lower respiratory tract infections are influenza and RSV. If hospital coordinators group children with bronchiolitis together in a room with multiple beds, they usually do so by RSV status. However, given that 1 of 3 children with RSV and almost 1 of 4 without RSV will have a coinfection, the effectiveness of grouping children with RSV-positive or RSV-negative bronchiolitis together is questionable, especially given that some of the coinfecting pathogens require droplet precautions rather than just contact precautions. Some<sup>36</sup> have suggested routinely using PCR to test for multiple respiratory viruses in critically ill children with lower respiratory tract infections, but the expense of using molecular testing for all children with severe bronchiolitis may not outweigh the potential benefits for the family and clinicians. Although one possibility would be to limit the testing to RSV and HRV, the ben-

efits of having a more complete picture of the infecting viruses—providing guidance about the potential severity of illness, possibly reducing antibiotic prescriptions,<sup>37</sup> and monitoring HRV-positive children closely for the development of asthma<sup>12-14</sup>—most likely do not outweigh the expense of the molecular testing for HRV.

Testing for HRV in a clinical setting may not be practical currently, but we recommend that HRV testing become more common in bronchiolitis research. To date, no one has rigorously or effectively defined subgroups of children with severe bronchiolitis who may respond differently to medications and/or have different clinical outcomes. Our results suggest that categorization by infectious pathogen (ie, RSV and HRV) may be necessary to most accurately interpret the findings of randomized trials and other bronchiolitis research, especially when using LOS as an outcome.<sup>38,39</sup> Trials that combine all children with clinical bronchiolitis into one group or categorize children by RSV status alone may obfuscate real associations. Therefore, bronchiolitis investigators may be missing clinically meaningful results by not including HRV status in their analyses.<sup>19</sup>

The present study has potential limitations. Polymerase chain reaction detects low amounts of virus in children, and HRV in particular is detected in up to 24% of children younger than 1 year without fever or other respiratory symptoms.<sup>40-43</sup> Therefore, it is conceivable that the HRV we detected is a “bystander” virus<sup>44</sup> and that these HRV infections are asymptomatic.<sup>40-43</sup> Alternatively, we may be detecting a recent infection from which the children were recovering and not the causative agent related to the hospitalization. Although it remains possible that some of the children with HRV were asymptomatic or in recovery, on the whole, these data suggest that, in children with severe bronchiolitis, HRV plays a central role in the clinical course and is not asymptomatic. Another issue is that the study participants were hospitalized in academic medical centers. Consequently, these results are not necessarily generalizable to community medical centers or when the children are outpatients. Furthermore, bronchiolitis is a clinical diagnosis<sup>8</sup> without a common international definition.<sup>8,45</sup> It is therefore possible that we included other respiratory disorders in this sample of children. However, when the data were restricted to resemble classic bronchiolitis, the results remained robust. Although the site teams enrolled 56% of the children and there were statistical differences in racial and ethnic groups, we do not believe that the level of enrollment or the statistical differences are clinically relevant or have provided biased results in this large multicenter study.

In summary, on the basis of these prospective, multicenter, multiyear data, we found that 1 in 3 children with severe bronchiolitis has a multiple virus infection, and we identified pathogen-based subgroups of children with different hospital LOS. Accordingly, we believe that these data raise questions about the effectiveness of RSV-based hospital cohorting practices. Moreover, severe bronchiolitis medication trials and other related research probably would benefit from inclusion of viral testing for both RSV and HRV so that lingering questions about differential effects by virus do not remain af-

ter the completion of otherwise rigorous trials. Most important, our data challenge current thinking that the infectious etiology of severe bronchiolitis does not affect short-term outcomes.

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**Author Affiliations:** Department of Medicine, Children’s Hospital Boston (Dr Mansbach), and Department of Emergency Medicine, Massachusetts General Hospital (Mss Sullivan and Espinola, Mr Forgey, and Dr Camargo), Harvard Medical School, Boston, Massachusetts; Departments of Molecular Virology and Microbiology and of Pediatrics, Baylor College of Medicine, Houston, Texas (Dr Piedra); Department of Pediatrics, Children’s National Medical Center, Washington, DC (Dr Teach); and Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania (Dr Clark).

**Correspondence:** Jonathan M. Mansbach, MD, Department of Medicine, Children’s Hospital Boston, 300 Longwood Ave, Main Clinical Bldg 9 S, Ste 9157, Boston, MA 02115 (jonathan.mansbach@childrens.harvard.edu).

**Author Contributions:** Drs Mansbach and Camargo had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Mansbach, Piedra, Teach, and Camargo. *Acquisition of data:* Mansbach, Piedra, Teach, Sullivan, Forgey, and Camargo. *Analysis and interpretation of data:* Mansbach, Piedra, Teach, Clark, Espinola, and Camargo. *Drafting of the manuscript:* Mansbach, Clark, and Camargo. *Critical revision of the manuscript for important intellectual content:* Mansbach, Piedra, Teach, Sullivan, Forgey, Clark, Espinola, and Camargo. *Statistical analysis:* Mansbach, Clark, Espinola, and Camargo. *Obtained funding:* Mansbach and Camargo. *Administrative, technical, and material support:* Sullivan, Forgey, and Camargo. *Study supervision:* Camargo.

**Group Members:** The MARC-30 site principal investigators were: Besh Barcega, MD, Loma Linda Medical Center, Loma Linda, California; John Cheng, MD, and Carlos Delgado, MD, Children’s Healthcare of Atlanta, Atlanta, Georgia; Haitham Haddad, MD, Rainbow Babies & Children’s Hospital, Cleveland, Ohio; Frank LoVecchio, MD, Maricopa Medical Center, Phoenix, Arizona; Charles G. Macias, MD, MPH, Texas Children’s Hospital, Houston; Eugene Mowad, MD, Akron Children’s Hospital, Akron, Ohio; Brian Pate, MD, Children’s Mercy Hospital, Kansas City, Missouri; Mark Riederer, MD, and Paul Hain, MD, Children’s Hospital at Vanderbilt, Nashville, Tennessee; M. Jason Sanders, MD, Children’s Memorial Hermann Hospital, Houston; Alan Schroeder, MD, Santa Clara Valley Medical Center, Santa Clara, California; Nikhil Shah, MD, and Dorothy Damore, MD, New York Presbyterian Hospital–Cornell, New York, New York; Michelle Stevenson, MD, Kosair Children’s Hospital, Louisville, Kentucky; Erin Stucky, MD, Rady Children’s Hospital, San Diego, California; Stephen Teach, MD, MPH, Children’s National Medical Center, Washington, DC; and Lisa Zaoutis, MD, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania.

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## REFERENCES

1. Carroll KN, Gebretsadik T, Griffin MR, et al. Increasing burden and risk factors for bronchiolitis-related medical visits in infants enrolled in a state health care insurance plan. *Pediatrics*. 2008;122(1):58-64.
2. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. *JAMA*. 1999;282(15):1440-1446.
3. Miron D, Srugo I, Kra-Oz Z, et al. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? *Pediatr Infect Dis J*. 2010;29(1):e7-e10. doi:10.1097/INF.0b013e3181c2a212.
4. Calvo C, Pozo F, García-García ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta Paediatr*. 2010;99(6):883-887.
5. Marguet C, Lubrano M, Gueudin M, et al. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One*. 2009;4(2):e4596. doi:10.1371/journal.pone.0004596.
6. Canducci F, Debiaggi M, Sampaolo M, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol*. 2008;80(4):716-723.
7. Stempel HE, Martin ET, Kuypers J, Englund JA, Zerr DM. Multiple viral respiratory pathogens in children with bronchiolitis. *Acta Paediatr*. 2009;98(1):123-126.
8. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics*. 2006;118(4):1774-1793.
9. Harris JA, Huskins WC, Langley JM, Siegel JD; Pediatric Special Interest Group of the Society for Healthcare Epidemiology of America. Health care epidemiology perspectives on the October 2006 recommendations of the Subcommittee on Diagnosis and Management of Bronchiolitis. *Pediatrics*. 2007;120(4):890-892.
10. Bush A, Thomson AH. Acute bronchiolitis. *BMJ*. 2007;335(7628):1037-1041.
11. Mansbach JM, McAdam AJ, Clark S, et al. Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. *Acad Emerg Med*. 2008;15(2):111-118.
12. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol*. 2005;116(3):571-577.
13. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003;111(1):66-71.
14. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*. 2008;178(7):667-672.
15. Beckham JD, Cadena A, Lin J, et al. Respiratory viral infections in patients with chronic, obstructive pulmonary disease. *J Infect*. 2005;50(4):322-330.
16. Knorr L, Fox JD, Tilley PA, Ahmed-Bentley J. Evaluation of real-time PCR for diagnosis of *Bordetella pertussis* infection. *BMC Infect Dis*. 2006;6:62. doi:10.1186/1471-2334-6-62.
17. Winchell JM, Thurman KA, Mitchell SL, Thacker WL, Fields BS. Evaluation of three real-time PCR assays for detection of *Mycoplasma pneumoniae* in an outbreak investigation. *J Clin Microbiol*. 2008;46(9):3116-3118.
18. Kusel MM, de Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007;119(5):1105-1110.
19. Lehtinen P, Ruohola A, Vanto T, Vuorinen T, Ruuskanen O, Jartti T. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol*. 2007;119(3):570-575.
20. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med*. 2002;165(9):1285-1289.
21. Simoes EA. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. *J Pediatr*. 2003;143(5)(suppl):S118-S126.
22. Carroll KN, Gebretsadik T, Griffin MR, et al. Maternal asthma and maternal smoking are associated with increased risk of bronchiolitis during infancy. *Pediatrics*. 2007;119(6):1104-1112.
23. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon- $\gamma$  response. *Pediatr Infect Dis J*. 2005;24(7):605-610.
24. Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB. Dual respiratory virus infections. *Clin Infect Dis*. 1997;25(6):1421-1429.
25. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. *Emerg Infect Dis*. 2003;9(3):372-375.
26. Semple MG, Cowell A, Dove W, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis*. 2005;191(3):382-386.
27. Regamey N, Kaiser L, Roiha HL, et al; Swiss Paediatric Respiratory Research Group. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J*. 2008;27(2):100-105.
28. Bont L, Heijnen CJ, Kavelaars A, et al. Local interferon- $\gamma$  levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J Infect Dis*. 2001;184(3):355-358.
29. Aberle JH, Aberle SW, Dworzak MN, et al. Reduced interferon- $\gamma$  expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med*. 1999;160(4):1263-1268.
30. Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon- $\lambda$  production in asthma exacerbations. *Nat Med*. 2006;12(9):1023-1026.
31. Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005;201(6):937-947.
32. Arnold R, König W. Respiratory syncytial virus infection of human lung endothelial cells enhances selectively intercellular adhesion molecule-1 expression. *J Immunol*. 2005;174(11):7359-7367.
33. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell*. 1989;56(5):839-847.
34. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF- $\kappa$ B-mediated transcription. *J Biol Chem*. 1999;274(14):9707-9720.
35. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. *Pediatr Allergy Immunol*. 2011;22(4):350-355.
36. Thomas NJ, Shaffer ML, Willson DF, Shih MC, Curley MA. Defining acute lung disease in children with the oxygenation saturation index. *Pediatr Crit Care Med*. 2010;11(1):12-17. doi:10.1097/PCC.0b013e3181b0653d.
37. Doan Q, Enarson P, Kissoon N, Klassen TP, Johnson DW. Rapid viral diagnosis for acute febrile respiratory illness in children in the emergency department. *Cochrane Database Syst Rev*. 2009;(4):CD006452.
38. Wainwright C, Altamirano L, Cheney M, et al. A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis. *N Engl J Med*. 2003;349(1):27-35.
39. Kuzik BA, Al-Qadhi SA, Kent S, et al. Nebulized hypertonic saline in the treatment of viral bronchiolitis in infants. *J Pediatr*. 2007;151(3):266-270.
40. Wright PF, Deatly AM, Karron RA, et al. Comparison of results of detection of rhinovirus by PCR and viral culture in human nasal wash specimens from subjects with and without clinical symptoms of respiratory illness. *J Clin Microbiol*. 2007;45(7):2126-2129.
41. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol*. 2006;78(5):644-650.
42. Fry AM, Lu X, Olsen SJ, et al. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. *PLoS One*. 2011;6(3):e17780.
43. Jansen RR, Wieringa J, Koekkoek SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol*. 2011;49(7):2631-2636.
44. Gerna G, Piralla A, Rovida F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol*. 2009;81(8):1498-1507.
45. Jartti T, Lehtinen P, Vuorinen T, Ruuskanen O. Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. *Pediatr Infect Dis J*. 2009;28(4):311-317.