

# Clinical Risk Factors Are More Relevant Than Respiratory Viruses in Predicting Bronchiolitis Severity

Silvia Ricart, MD,<sup>1\*</sup> Maria Angeles Marcos, MD, PhD,<sup>2</sup> Marta Sarda, MD,<sup>1</sup> Andres Anton, MD,<sup>2</sup> Carmen Muñoz-Almagro, MD, PhD,<sup>3</sup> Tomas Pumarola, MD, PhD,<sup>2</sup> Marti Pons, MD,<sup>4</sup> and Juan Jose Garcia-Garcia, MD, PhD<sup>1</sup>

**Summary.** Background: The role of respiratory viruses in the pathogenesis of bronchiolitis was re-evaluated with the use of molecular methods such as PCR for virus detection. Whether specific viruses or the classical clinical risk factors are more important in determining severe bronchiolitis is not well established. Aim: To analyze the specific viruses and clinical variables that can predict severe bronchiolitis at admission. Methods: Nasopharyngeal aspirates were prospectively collected from 484 children <12 months admitted to the pediatrics ward or PICU at University Hospital Sant Joan de Déu (Barcelona, Spain) for bronchiolitis from October 2007 to October 2008. Clinical and demographic data were collected. Sixteen respiratory viruses were studied using PCR. Severity was assessed with a bronchiolitis clinical score (BCS). Results: Four hundred ten infants that tested positive for respiratory viruses were analyzed. Mixed viral infections did not increase the severity of the disease. Rhinovirus was associated with severe BCS in univariate analysis ( $P = 0.041$ ), but in the multivariate logistic regression including viruses and clinical data only bronchopulmonary dysplasia (OR 7.2; 95% CI 1.2–43.3), congenital heart disease (OR 4.7; 95% CI 1.1–19.9), prematurity (OR 2.6; 95% CI 1.3–5.1), and fever (OR 1.8, 95% CI 1.1–3.1) showed statistical significance for predicting severe BCS. Conclusions: Classical clinical risk factors have more weight in predicting a severe BCS in infants with acute bronchiolitis than the involved viruses. **Pediatr Pulmonol.** 2013; 48:456–463. © 2012 Wiley Periodicals, Inc.

**Key words:** viral bronchiolitis; risk factors; disease severity; multivariate predictors.

**Funding source:** Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (ISCIII, FIS 05/0063); Fundació Sant Joan de Deu (AFR 2007/02); and Agencia de Gestio d'Ajuts Universitaris i de Recerca (AGAUR, 2009/SGR00136).

## INTRODUCTION

Acute respiratory infections are a major cause of hospitalization in pediatric populations, especially in the early years of life.<sup>1</sup> Acute bronchiolitis is one of the best-characterized respiratory diseases in infants; around 2–3% of children younger than 1 year of age are admitted to hospital with bronchiolitis,<sup>2</sup> and up to 14% of these patients require admission to pediatric intensive care units (PICU).<sup>3,4</sup> Human respiratory syncytial virus (HRSV) is the most commonly identified virus, detected in up to 60–70% of hospitalized infants.<sup>5,6</sup> Other respiratory viruses, including parainfluenza virus, coronavirus, adenovirus, rhinovirus (HRV), and, recently, human metapneumovirus (HMPV)<sup>7,8</sup> and human bocavirus (HBoV),<sup>9</sup> have been described as significant causes of acute bronchiolitis.

High-risk groups for severe bronchiolitis are infants younger than 6 weeks old with predisposing conditions such as prematurity and congenital heart disease (CHD).<sup>10,11</sup> Over the past decade, with the development

Additional supporting information may be found in the online version of this article.

<sup>1</sup>Department of Pediatrics, Hospital Universitari Sant Joan de Déu, Barcelona, Spain.

<sup>2</sup>Department of Microbiology, Barcelona Centre for International Health Research (CRESIB, Hospital Clínic - Universitat de Barcelona), Barcelona, Spain.

<sup>3</sup>Department of Microbiology, Hospital Universitari Sant Joan de Déu, Barcelona, Spain.

<sup>4</sup>Pediatric Intensive Care Unit, Hospital Universitari Sant Joan de Déu, Barcelona, Spain.

Conflict of interest: None.

\*Correspondence to: Silvia Ricart, MD, Passeig Sant Joan de Déu, 2 Esplugues de Llobregat, Barcelona 08950, Spain.  
E-mail: sricart@hsjdbcn.org

Received 26 October 2011; Accepted 16 April 2012.

DOI 10.1002/ppul.22633

Published online 4 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

of new molecular techniques for virus detection, the role of various respiratory viruses in the pathogenesis of acute bronchiolitis has been re-evaluated. HRV, the most prevalent cause of upper respiratory infection in older children and adults, is increasingly recognized as an important agent causing acute bronchiolitis. Its role in the development of acute respiratory tract infection is controversial because it can be detected in up to 15% of asymptomatic individuals.<sup>12</sup> Moreover, it is associated with a more severe course of the disease.<sup>13</sup> In addition, viral coinfections have also been related with severe bronchiolitis.<sup>9,14,15</sup>

Whether the traditional clinical risk factors or data concerning infection by specific viruses have more weight in the development of a severe course in infants affected by acute bronchiolitis remains unknown. The aim of this study was to assess the clinical factors and the viruses involved in cases of severe bronchiolitis, defining severity according to the respiratory burden using a bronchiolitis clinical score (BCS). Secondly, we aimed to investigate the clinical factors and specific respiratory viruses that can be used to predict a severe outcome of the disease at admission.

## MATERIALS AND METHODS

### Study Population

Prospective study in infants <12 months with acute bronchiolitis hospitalized from October 2007 to October 2008 in the pediatric ward or the PICU of Hospital Sant Joan de Deu, a tertiary university hospital located in Barcelona, Spain. Bronchiolitis was diagnosed according to the presence of the first acute respiratory tract infection characterized by respiratory distress (tachypnea, use of accessory muscles), cough, widespread crackles, wheezing or both, associated with signs of viral infection (coryza).<sup>2</sup> Exclusion criteria were underlying chronic pulmonary disease other than pulmonary bronchodysplasia (BPD) (in order not to exclude patients born prematurely, who are known to be at risk for severe bronchiolitis); recurrent (more than one) wheezing episodes; apnea secondary to a known disease (gastroesophageal reflux demonstrated by pH-metry or esophago-gastrointestinal transit, metabolic or neurologic disease); and respiratory symptoms due to bronchoaspiration.

Patients were admitted to the hospital if one of the following criteria was present: pulse oximetry less than 92% on room air persistently during the stay in emergency room (minimum 1 hr), poor feeding with risk of dehydration, apnea, moderate bronchiolitis defined as a BCS<sup>16,17</sup> equal to or greater than six points, known cardiorespiratory disease (CHD or BPD) or young age of the child (less than 4 weeks). The BCS is a modified Wood-Downes score that assesses auscultation,

transcutaneous hemoglobin saturation, respiratory effort, and heart rate and respiratory rate according to age, with a score ranging between 0 and 16 points (E-Fig. 1). Criteria for PICU admission were acute respiratory failure with severe hypoxemia (pulse oximetry <90% with oxygen supplementation greater than 40%), sepsis (defined according to International Consensus Conference on Pediatric Sepsis, 2005<sup>18</sup>), frequent apnea requiring vigorous stimulation, severe bronchiolitis or pneumonia, acidosis (pH less than 7.1), or rapidly progressing disease. Severe bronchiolitis was defined by a maximum BCS equal to or greater than 11 points during the illness according to our hospital bronchiolitis clinical practice guidelines.

Informed consent was obtained from the patients' parents. The ethics committees of both the Hospital Clinic and Hospital Sant Joan de Déu approved the study protocol.

### Recorded Data

Demographic, epidemiologic, clinical and laboratory data were obtained using a standardized questionnaire. Recorded variables included age, sex, birth history (gestational age and birth weight), need for neonatal admission, use of palivizumab prophylaxis (which was prescribed according to American Academy of Pediatrics recommendations 2003<sup>19</sup>), personal history of atopy, family history of asthma or atopy in first-degree relatives, and presence of underlying chronic conditions. Hemodynamically significant CHD (defined either by the use of medication to control congestive heart failure, infants with moderate to severe pulmonary hypertension or with cyanotic heart disease),<sup>19</sup> BPD defined by Jobe and Bancalari,<sup>20</sup> polymalformative syndrome or chromosomopathy, or severe neurologic impairment were considered significant chronic conditions. In the emergency room weight, vital signs (heart rate, respiratory rate, axillary temperature, transcutaneous hemoglobin saturation), severity (according to BCS, E-Fig. 1), days of coryza and days of respiratory effort prior to admission and history of fever registered by the caretakers 48 hr prior to admission were recorded. In case of septic-appearance infants without fever in axillary measurement, rectal temperature was taken and registered in case of discrepancy. During inpatient management the following data were recorded: days of oxygen therapy, maximum fraction of inspired oxygen (FiO<sub>2</sub>) needed, tests performed and their results (chest X-ray, laboratory and microbiological data), days of nasogastric feeding tube, need for and length of respiratory support (either by mechanical ventilation or non-invasive ventilation), need for and length of stay in the PICU, diagnosis at discharge and length of hospitalization. The radiological findings were reviewed by

radiologists and classified according to the severity into: peribronchial infiltrates and hyperinflation without consolidation or atelectasis, peribronchial infiltrates and hyperinflation with mild-to-moderate consolidation or atelectasis, extensive consolidation or atelectasis with or without hyperinflation.<sup>21</sup> Because the disease could worsen after admission, the BCS was assessed daily and the highest score observed during the hospitalization was retained for determination of the severity of the disease (maximum BCS).

## Detection of Respiratory Viruses

### Direct Antigen Detection

A nasopharyngeal aspirate to study respiratory viruses was collected in the emergency room or within the first 24 hr of admission. An aliquot of the sample was used to perform a rapid immunochromatography test to detect HRSV (Clearview RSV, Unipath) in the emergency lab, and afterwards the sample was delivered to the Laboratory of Microbiology in the Hospital Clinic of Barcelona, where it was processed for PCR virus detection.

### Detection of Viral RNA/DNA by Molecular Methods

Total nucleic acids were extracted from 200  $\mu$ l of fresh specimen and eluted in 25  $\mu$ l of RNase-free elution buffer using NucliSense easyMAG (bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions. The lysis buffer included 500 molecules of the cloned amplified product used as internal control in each reaction tube and excluded false-negative results due to non-specific inhibitors or extraction failure. Two independent multiplex nested RT-PCR assays were performed as described by Coiras et al.<sup>22,23</sup> A first multiplex RT-PCR assay detected influenza A, B, and C viruses, human adenovirus, and HRSV A and B subtypes. Another RT-PCR assay studied human parainfluenza viruses 1, 2, 3, 4, human coronavirus 229E and OC43, HRV, and human enterovirus. In order to detect HBoV and HMPV subtypes A and B a real-time multiplex RT-PCR was used. A set of primers and probe for HBoV specific to the NS-1 gene (forward primer: 5'-TGCACTGATTGCGGTGGTT-3'; reverse primer: 5'-TGCGCGATCAGTGTACATAGAA-3'; probe: 5'-(VIC)-CCTTCCTCATAGTGCTGAC-(MGB)-3') was designed using Primer Express software (Applied Biosystems, Foster City, CA). The sets of primers and probes specific to the fusion gene of both subtypes of HPMV previously described<sup>24</sup> were used, labeled with 5'-(6-FAM) and 3'-MGB. The real-time RT-PCR reactions were carried out using the Mx3000P (Stratagene, La Jolla, CA).

## Study of Bacterial Infection

Urine cultures and blood cultures were taken in cases of infants less than 3 months old with fever (axillary or rectal temperature  $\geq 38^{\circ}\text{C}$ ), or infants of any age with temperature equal to or greater than  $39^{\circ}\text{C}$  or with septic appearance. Lumbar puncture was done in febrile infants <1 month or older with suspected sepsis. In the PICU setting broncho-alveolar lavage and culture was done in cases of suspicion of bacterial coinfection, and central line catheters were cultured in patients with suspected nosocomial infection. Bacterial/yeast coinfection was defined as the growing of a pathogenic microorganism in blood, urine, cerebrospinal fluid, or broncho-alveolar lavage fluid.

## Study Groups

In order to assess the variables related to a severe disease in terms of respiratory symptoms, patients were divided into two groups for comparison: children who reached a maximum BCS equal to or greater than 11 points at admission or during hospital stay were classified as severe. Children with maximum BCS less than 11 points were classified into the non-severe group.

## Statistical Analysis

Descriptive statistics for discrete variables were reported in terms of absolute frequencies and percentages, and data comparison was made using Chi-square test or Fisher exact test when appropriate. Continuous non-normal distributed variables were described as median, first and third quartile. Bivariate associations were assessed with Student's *t*-tests for normally distributed variables and Mann-Whitney *U*-test for non-normally distributed variables. *P*-values <0.05 were considered significant.

Multivariate logistic regression analyses were used to evaluate the risk factors for severe disease. Initially, univariate models were performed introducing as independent variables age, gestational age, weight, personal and family history of atopy, presence of underlying chronic condition (BPD, CHD, significant chromosomalopathy or polymalformative syndrome, or severe neurologic disability), presence of fever at admission or the previous 48 hr, days of coryza and days of respiratory effort prior to admission, and all the 16 respiratory viruses tested. Subsequently, the variables with a significant association ( $P < 0.1$ ) in the univariate models and variables known as potential confounders (palivizumab immunoprophylaxis and HRSV infection) were introduced in multivariate models with a step-wise approach to eliminate the possibility of mutual confounding and interaction. The risk was expressed as odds ratio (OR) with 95% confidence intervals (95% CI). Multivariate

**TABLE 1—Demographic Characteristics, Clinical Course, and Virus Detection in the Infants Included With Bronchiolitis (n = 410)**

Demographic characteristics	
Gender, n (%)	
Male	237 (57.8%)
Females	173 (42.2%)
Age, median (IQR), months	1.9 (1.1–3.9)
Weight at admission, median (IQR), kg	4.8 (3.9–6.2)
Prematurity (gestational age <37 weeks), n (%)	67 (16.3%)
Underlying chronic conditions, n (%)	34 (8.3%)
CHD, n (%) <sup>1</sup>	12 (35.3%)
BPD, n (%) <sup>1</sup>	10 (29.4%)
Polymalformative syndrome or chromosomopathy, <sup>2</sup> n (%) <sup>1</sup>	8 (23.5%)
Severe neurologic impairment, n (%) <sup>1</sup>	4 (11.8%)
Disease characteristics	
Admission	
PICU, n (%)	58 (14.1%)
Pediatric ward, n (%)	352 (85.9%)
Length of stay, median (IQR), days	4.0 (3.0–7.0)
Supplemental oxygen, n (%)	327 (79.8%)
Days of oxygen use, median (IQR), days	3 (1–5)
Apnea, n (%)	24 (5.9%)
Fever, <sup>3</sup> n (%)	168 (41%)
Radiologic evaluation, n (%)	
No performed	221 (53.9%)
No pathologic findings or peribronchial infiltrates without consolidation or atelectasis	79 (19.3%)
Peribronchial infiltrates with mild-to-moderate consolidation or atelectasis	76 (18.5%)
Extensive consolidation or atelectasis	34 (8.3%)
BCS at admission, median (IQR), points	7 (5–9)
Highest BCS during the inpatient management, median (IQR), points	8 (6–10)

IQR, interquartile range; BPD, bronchopulmonary dysplasia; CHD, congenital heart disease; PICU, pediatric intensive care unit; BCS, bronchiolitis clinical score.

<sup>1</sup>Numbers in parentheses represent the percentage calculated for patients with an underlying chronic illness.

<sup>2</sup>Two patients with Down syndrome (sd), craneofacial malformation with facial and subglottic angioma, polymalformative sd without specific diagnosis (Pierre–Robin sequence, arthrogryposis, thrombocytopenia, interventricular defect, ventriculomegaly), CHARGE sd, Branchio-otic (BO) sd, Rubinstein–Taybi sd, and polymalformative sd without specific diagnosis (anorectal malformation, congenital diaphragmatic hernia, aortic arch hypoplasia).

<sup>3</sup>Temperature  $\geq 38^{\circ}\text{C}$  at admission or the 48 hr before.

odds ratios and 95% CIs that did not include 1 were considered significant. All the analyses were performed using PASW Statistical 17.0.2 software.

## RESULTS

### Total Sample

Five-hundred sixty-three patients were admitted for bronchiolitis during the study period; 53 of them were excluded for meeting at least one of the exclusion criteria, 23 because of insufficient nasopharyngeal aspirate sample and 3 for not meeting the standardized admission criteria (admitted for parental discomfort or lack of follow-up). Four hundred eighty-four hospitalized infants with bronchiolitis were included. Four hundred ten (84.7%) of the infants tested positive for virus detection, 221 (53.9%) for a single virus and 189 (46.1%) for more than one virus (E-Fig. 2).

### Characteristics of the Patients in the Study Group and Virus Distribution

In order to investigate the role of the respiratory viruses in the clinical course of the patients with bronchiolitis, only the infants with a positive result for virus detection were included in the analysis (total n = 410). Patient demographics and disease characteristics are shown in Table 1. When the viruses in coinfection were assessed separately (Table 2), a total of 666 viruses were detected in the 410 positive samples. The most common pathogen was HRSV (287 virus detected; 43.1%), followed by HRV (125; 18.8%), HBoV (119; 17.9%), and human adenovirus (52; 7.8%). Among infants with HRSV bronchiolitis, 2.5% had an underlying chronic illness and 12.1% had a gestational age <37 weeks, contrasting with infants with non-HRSV bronchiolitis whose proportions were 20.8% ( $P = 0.001$ ) and 25.4% ( $P < 0.0005$ ), respectively. Palivizumab

**TABLE 2—Virus Detected (n = 666 Viruses) in the 410 Nasopharyngeal Aspirates Positive for Virus Detection**

Virus	n (%)
HRSV subtype A	233 (35.0)
HRSV subtype B	54 (8.1)
Rhinovirus	125 (18.8)
Human bocavirus	119 (17.9)
Adenovirus	52 (7.8)
Human metapneumovirus	26 (3.9)
Influenza A virus	14 (2.1)
Enterovirus	9 (1.4)
Coronavirus 229E	8 (1.2)
Coronavirus OC43	6 (0.9)
Parainfluenza 2	6 (0.9)
Parainfluenza 4	6 (0.9)
Parainfluenza 3	5 (0.7)
Parainfluenza 1	3 (0.4)
Influenza B virus	0

immunoprophylaxis was up to date in 96.6% of the patients in whom it was indicated.

The presence of fever at admission or the 48 hr before showed a statistically significant association with finding X-ray consolidation or atelectasis ( $P < 0.0005$ ): of 168 infants with fever, 76 (45.2%) had mild-to-moderate X-Ray opacities and 34 (20.2%) extensive consolidation or atelectasis.

### Bacterial Coinfection

Concomitantly with the viral respiratory infection, 14 patients had bacterial/yeast infections (3.4% of the patients): 7 patients with urinary tract infections (2 *Escherichia coli*, 3 *Klebsiella oxytoca*, 1 *Streptococcus faecalis*, and 1 *Candida albicans*), 2 patients with positive blood culture (*Streptococcus pneumoniae* and *Neisseria meningitidis*), and 5 patients with positive culture of the protected broncho-alveolar lavage (3 *S. pneumoniae*, 1 *Pseudomonas aeruginosa*, 1 *Enterobacter cloacae*). In 11 infants the bacterial coinfection was detected in the PICU (only 4 of them were nosocomial infections), while the other 3 patients remained in the pediatric ward.

### Analysis of the Factors Related to Severe Bronchiolitis

Eighty-two (20%) of the 410 patients experienced severe BCS during the hospital stay. Patients in the severe group had an increased mean length of stay compared with the non-severe group (11.2 days (standard deviation 10.4) vs. 4.3 days (SD 3.1),  $P < 0.005$ ), increased days of oxygen therapy (9.0 days (SD 8.2) vs. 2.6 days (SD 2.5),  $P < 0.005$ ), increased maximum FiO<sub>2</sub> required (mean FiO<sub>2</sub> 43% (SD 18.4) vs. 26% (SD 7.1),  $P < 0.005$ ), increased days with naso-gastric feeding tub (6.7 (range 0–58) vs. 0.7 (range 0–17) days,

$P < 0.005$ ), greater PICU admission (50% vs. 3.4%,  $P < 0.005$ ), and greater need for respiratory support, either by non-invasive ventilation (43.9% vs. 2.5%,  $P < 0.005$ ) or mechanical ventilation (32.9% vs. 0.6%,  $P < 0.005$ ). For patients in the non-severe group who required PICU admission and respiratory support this was due to the development of apnea. Twenty-one infants went into the PICU for apnea, 11 from non-severe and 10 from severe group.

The presence of bacterial coinfection also correlated with severity: 66.7% of the infants coinfecting by bacteria had a severe BCS ( $P < 0.005$ ).

### Univariate Analysis

Univariate analysis with all the variables recorded at admission revealed that age ( $P = 0.037$ ), fever ( $P = 0.012$ ), premature birth ( $P = 0.009$ ), BPD ( $P = 0.006$ ), CHD ( $P = 0.05$ ), and severe neurologic impairment ( $P = 0.002$ ) were associated with severe BCS. In the univariate analyses of the main viruses (HRSV, HMPV, HBoV, HRV) and viral coinfections, only HRV infection was associated with a severe BCS ( $P = 0.041$ ). Dual infection by HRV and HRSV and other viral coinfections did not reach statistical significance. Table 3 shows the results of the univariate analysis. Bacterial coinfection was not introduced into univariate analysis because these data are not available at patient admission.

### Multivariate Logistic Regression Analysis

After introducing the significant variables and the potential confounders into univariate analysis, the adjusted final model for severe disease according to BCS included BPD (OR 7.2; 95% CI 1.2–43.3;  $P = 0.031$ ), hemodynamically significant CHD (OR 4.7; 95% CI 1.1–19.9;  $P = 0.038$ ), gestational age <37 weeks (OR 2.6; 95% CI 1.3–5.1;  $P = 0.005$ ), and temperature >38°C (OR 1.8; 95% CI 1.1–3.1;  $P = 0.027$ ). HRV, HRSV, and age (with a  $P = 0.055$ ) were excluded from the final model. E-Table 1 shows the results of the multivariate logistic regression model with the confidence intervals.

### DISCUSSION

The development of the molecular techniques for virus detection has shown the importance of other viruses in addition to HRSV in the pathogenesis of acute bronchiolitis. This study analyses, for the first time, the relative importance of clinical and virological data in predicting bronchiolitis outcome. Our results show that, in our environment, the clinical data obtained at admission are more relevant than the specific infecting virus in determining the risk of severe

**TABLE 3—Univariate Analysis Assessing Risk Factors for Severe Bronchiolitis (According to BCS  $\geq 11$  Points) and Differences Among Severe and Non-Severe Group**

	Non-severe group (n = 328)	Severe group (n = 82)	P-value
Rhinovirus, n (%)	91 (27.7)	32 (39.0)	0.041*
Age (months), median (IQR)	1.8 (1.0–3.5)	2.7 (1.1–5.0)	0.037*
Temperature $\geq 38^{\circ}\text{C}^{\dagger}$ , n (%)	123 (37.5)	44 (53.7)	0.012*
Premature birth, n (%)	41 (12.5)	21 (25.6)	0.009*
BPD, n (%)	4 (1.2)	6 (7.3)	0.006*
CHD, n (%)	7 (2.1)	5 (6.1)	0.050*
Severe neurologic impairment, n (%)	0	4 (4.9)	0.002*
HRSV, n (%)	228 (69.5)	52 (63.4)	0.147
HMPV, n (%)	19 (5.8)	7 (8.5)	0.269
HBoV, n (%)	97 (29.6)	22 (26.8)	0.291
Viral coinfection, n (%)	155 (47.3)	34 (41.5)	0.414
Dual infection HRSV–HRV, n (%)	44 (13.4)	12 (14.6)	0.860
Dual infection HRSV–HMPV, n (%)	3 (0.9)	1 (1.2)	1.0
Atopy, n (%)	59 (18.0)	18 (22.0)	0.139
Chromosomopathy, n (%)	5 (1.5)	3 (3.7)	0.208
Weight (kg), mean (IQR)	4.7 (3.9–6.0)	5.0 (3.8–6.7)	0.395
Days of coryza prior to admission, mean (IQR)	3.5 (2.0–5.0)	3.0 (2.0–5.0)	0.515

BCS, bronchiolitis clinical score; IQR, interquartile range; BPD, bronchopulmonary dysplasia; CHD, congenital heart disease; HRSV, human respiratory syncytial virus; HMPV, human metapneumovirus; HBoV, human bocavirus.

Percentages refer to the column group.

<sup>†</sup>At admission or the 48 hr before.

\*Statistical significant results.

disease. The strength of this study is that we prospectively enrolled only hospitalized infants less than 12 months of age with a first acute episode of bronchiolitis, excluding all the patients with recurrent wheezing, whereas other studies retrospectively investigated the results of virological examinations in patients of different ages and with various respiratory diseases.<sup>25</sup> We also studied all the significant respiratory viruses that are currently associated with bronchiolitis. As a result, our study offers a homogeneous and well-characterized cohort to examine the natural history and risk factors of acute bronchiolitis.

Some studies have defined severity according to the need for PICU admission.<sup>9,14,15</sup> Because PICU admission criteria can differ between centers (according individual practice guidelines or PICU availability), we analyzed severity based on BCS, a reproducible clinical score which allows classification of the patients according to the respiratory findings on physical examination and oxygen saturation, reflecting the severity in terms of respiratory burden. This BCS correlates with other variables involved with severity such as the length of stay, need for supplementary oxygen, and need for PICU admission and respiratory support. In the univariate logistic regression analysis, HRV was the only virus found to be a risk factor for severe BCS in acute bronchiolitis. This finding is in accordance with the report of Papadopoulos et al.,<sup>13</sup> in which the presence of HRV in infants with bronchiolitis was found to increase by 5.6-fold the risk for severe disease (defined by a clinical score at admission above the 50th percentile). Other

reports have also described HRV as an important pathogen in the lower respiratory tract, implicated in severe respiratory distress in children with underlying chronic conditions<sup>26</sup> and significantly associated with an increase in the rate of hospitalizations, especially in patients with a history of wheezing or asthma.<sup>27</sup>

In our series the proportion of ex-preterm and infants with underlying chronic illness is lower in HRSV bronchiolitis than in bronchiolitis caused by other viruses, which is in accordance with previous studies that described how the majority of children with HRSV infection in developed countries had no underlying medical conditions.<sup>28,29</sup> The high palivizumab prophylaxis rate in our environment could play a role in the lower hospitalization rate for HRSV bronchiolitis in high-risk infants.<sup>30</sup> Some studies have reported an increased risk for severe bronchiolitis in dual viral infection,<sup>9,14,15</sup> or even in some specific viral infections such as HRSV–HMPV coinfection.<sup>31–33</sup> Our results are in accordance with other series that did not find an increase in severity in patients with viral coinfections.<sup>34–37</sup> Moreover, the high frequency of viral coinfections in our study, as also found in previous works, indicates that infection with multiple viruses in infants is a common situation that does not change the clinical course of bronchiolitis.

The multivariate logistic regression model showed that BPD was the strongest predictor of severe score, followed by hemodynamically significant CHD and prematurity, all well-known risk factors for severe HRSV bronchiolitis according to previous reports.<sup>3,10,38,39</sup> The

last predictor in our analysis was fever at admission, which was significantly associated with X-ray infiltrates in our series. This finding has also been described in previous works, where pulmonary consolidation on chest X-ray was associated with severe disease and need for respiratory support,<sup>3</sup> even in previously healthy infants.<sup>40</sup>

Most of the studies regarding risk factors for severe bronchiolitis have been conducted in children infected by HRSV, but to our knowledge no studies have analyzed the risk factors for all patients with bronchiolitis and the relative importance of viruses and traditional risk factors. With our results an interesting conclusion can be drawn, which is that, beside the implicated virus, the classical risk factors for HRSV bronchiolitis can be extended to include bronchiolitis caused by other viruses. This observation is important in planning protocols for bronchiolitis management; although palivizumab prophylaxis is administered to high-risk patients for severe HRSV bronchiolitis, most of them continue to be at risk for severe bronchiolitis from other viruses. Efforts to educate caretakers on how to prevent viral infections in high-risk patients should continue, especially where moderate preterms, infants with BPD and CHD are concerned. Additionally, HRV should be taken into account. In our area, where universal HRSV prophylaxis for high-risk patients HRV is associated with severe cases when clinical factors are not considered, routine screening for HRV at admission may be proposed in order to identify infants at risk for severe disease and to separate the hospitalized patients into cohorts so as to avoid nosocomial infection.

In evaluating the findings of the present study, some limitations must be highlighted. First, this study was conducted only during one season. Severity of infection by a virus can fluctuate in different seasons, as can the pattern of circulating viruses in the community, which may explain the differences in severity by specific viruses found in this and other reports. Also, the high palivizumab prophylaxis rate in our population probably leads to underestimation of the role of HRSV in high-risk infants. Another limitation is that BCS is not yet a validated instrument for bronchiolitis scoring. Nevertheless, none of the available scoring systems for bronchiolitis has been validated neither has all tests characteristics studied (reliability, responsiveness, reproducibility). BCS is implemented in the daily clinical practice in our institution for 15 years and shows good correlation with other outcomes of severity (length of hospital stay, days of oxygen therapy and of nasogastric feeding tub, FiO<sub>2</sub>, greater PICU admission and need for respiratory support). The use of different bronchiolitis scoring systems is a common limitation of most articles in this field because it difficult to compare with other studies.

Finally, pathogen detection in nasopharynx may not accurately correlate with infection of the lower respiratory tract, and in addition some detected viruses might reflect late shedding after a past infection. Nevertheless, this limitation is common to other similar reports. The use of real-time PCR for viral load quantification<sup>41</sup> may annul this limitation in upcoming projects.

In summary, we analyzed the relative importance of clinical factors and specific viruses in predicting severe bronchiolitis. Our data suggest that, although HRV seems to be related to increased risk of severe disease in univariate analysis, traditional clinical risk factors have more specific weight in predicting bronchiolitis outcome.

## ACKNOWLEDGMENTS

This study was funded by a grant from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (ISCIII, grant number FIS 05/0063), the Fundació Sant Joan de Deu (AFR 2007/02) and the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR, 2009/SGR00136).

## REFERENCES

1. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980–1996. *JAMA* 1999;282:1440–1446.
2. Smyth RL, Openshaw PJ. Bronchiolitis. *Lancet* 2006;368:312–322.
3. Wang EE, Law BJ, Stephens D. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. *J Pediatr* 1995;126:212–219.
4. Willson DF, Landrigan CP, Horn SD, Smout RJ. Complications in infants hospitalized for bronchiolitis or respiratory syncytial virus pneumonia. *J Pediatr* 2003;143:S142–S149.
5. Calvo C, Pozo F, Garcia-Garcia ML, Sanchez M, Lopez-Valero M, Perez-Brena P, Casas I. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta Paediatr* 2010;99:883–887.
6. Henrickson KJ, Hoover S, Kehl KS, Hua W. National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatr Infect Dis J* 2004;23:S11–S18.
7. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;7:719–724.
8. Ordas J, Boga JA, Alvarez-Arguelles M, Villa L, Rodriguez-Dehli C, de Ona M, Rodriguez J, Melon S. Role of metapneumovirus in viral respiratory infections in young children. *J Clin Microbiol* 2006;44:2739–2742.
9. Midulla F, Scagnolari C, Bonci E, Pierangeli A, Antonelli G, De Angelis D, Berardi R, Moretti C. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. *Arch Dis Child* 2010;95:35–41.
10. Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. *J Pediatr* 2003;143:S112–S117.

11. Purcell K, Fergie J. Driscoll Children's Hospital respiratory syncytial virus database: risk factors, treatment and hospital course in 3308 infants and young children, 1991 to 2002. *Pediatr Infect Dis J* 2004;23:418–423.
12. Peltola V, Waris M, Osterback R, Susi P, Hyypia T, Ruuskanen O. Clinical effects of rhinovirus infections. *J Clin Virol* 2008; 43:411–414.
13. Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med* 2002;165:1285–1289.
14. McNamara PS, Flanagan BF, Smyth RL, Hart CA. Impact of human metapneumovirus and respiratory syncytial virus coinfection in severe bronchiolitis. *Pediatr Pulmonol* 2007;42: 740–743.
15. Richard N, Komurian-Pradel F, Javouhey E, Perret M, Rajoharison A, Bagnaud A, Billaud G, Vernet G, Lina B, Floret D, Paranhos-Baccala G. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. *Pediatr Infect Dis J* 2008;27:213–217.
16. Puigarnau Vallhonrat R, Miranda Vives A, Sánchez Etxaniz J. Manejo de la bronquiolitis en urgencias. In: Benito J, Luaces C, Mintegui S, Pou J, editors. *Tratado de urgencias en pediatría*. Madrid: Ergon; 2005. pp 300–305.
17. Rovira N, Ricart S, Curcoy AI, Trenchs V, Ribó JL, Luaces C. Indicació de la radiografia de tòrax en infants ingressats per bronquiolitis: sempre? *Pediatr Catalana* 2007;67:221–224. Available at: [http://www.scpediatría.cat/pediatrcatalana/index.php?doc=docs/2007/pc5/torig\\_radiografia.pdf](http://www.scpediatría.cat/pediatrcatalana/index.php?doc=docs/2007/pc5/torig_radiografia.pdf)
18. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International Pediatric Sepsis Consensus Conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med* 2005;6:2–8.
19. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. Revised indications for the use of palivizumab and respiratory syncytial virus immune globulin intravenous for the prevention of respiratory syncytial virus infections. *Pediatrics* 2003;112:1442–1446.
20. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;163:1723–1729.
21. Papoff P, Moretti C, Cangiano G, Bonci E, Roggini M, Pierangeli A, Scagnolari C, Antonelli G, Midulla F. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. *Acta Paediatr* 2011;100:e17–e23.
22. Coiras MT, Perez-Brena P, Garcia ML, Casas I. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. *J Med Virol* 2003;69:132–144.
23. Coiras MT, Aguilar JC, Garcia ML, Casas I, Perez-Brena P. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. *J Med Virol* 2004;72:484–495.
24. Kuypers J, Wright N, Corey L, Morrow R. Detection and quantification of human metapneumovirus in pediatric specimens by real-time RT-PCR. *J Clin Virol* 2005;33:299–305.
25. Bonzel L, Tenenbaum T, Schrotten H, Schildgen O, Schweitzer-Krantz S, Adams O. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;27:589–594.
26. Kim JO, Hodinka RL. Serious respiratory illness associated with rhinovirus infection in a pediatric population. *Clin Diagn Virol* 1998;10:57–65.
27. Miller EK, Lu X, Erdman DD, Poehling KA, Zhu Y, Griffin MR, Hartert TV, Anderson LJ, Weinberg GA, Hall CB, Iwane MK, Edwards KM, New Vaccine Surveillance Network. Rhinovirus-associated hospitalizations in young children. *J Infect Dis* 2007;195:773–781.
28. Garcia CG, Bhore R, Soriano-Fallas A, Trost M, Chason R, Ramilo O, Mejias A. Risk factors in children hospitalized with RSV bronchiolitis versus non-RSV bronchiolitis. *Pediatrics* 2010;126:e1453–e1460.
29. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, Auinger P, Griffin MR, Poehling KA, Erdman D, Grijalva CG, Zhu Y, Szilagyi P. The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009; 360:588–598.
30. Wright M, Piedimonte G. Respiratory syncytial virus prevention and therapy: past, present, and future. *Pediatr Pulmonol* 2011; 46:324–347.
31. Semple MG, Cowell A, Dove W, Greensill J, McNamara PS, Halfhide C, Shears P, Smyth RL, Hart CA. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 2005;191:382–386.
32. Foulongne V, Guyon G, Rodiere M, Segondy M. Human metapneumovirus infection in young children hospitalized with respiratory tract disease. *Pediatr Infect Dis J* 2006;25:354–359.
33. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. *Emerg Infect Dis* 2003;9:372–375.
34. Wilkesmann A, Schildgen O, Eis-Hubinger AM, Geikowski T, Glatzel T, Lentze MJ, Bode U, Simon A. Human metapneumovirus infections cause similar symptoms and clinical severity as respiratory syncytial virus infections. *Eur J Pediatr* 2006;165: 467–475.
35. Garcia-Garcia ML, Calvo C, Martin F, Perez-Brena P, Acosta B, Casas I. Human metapneumovirus infections in hospitalised infants in Spain. *Arch Dis Child* 2006;91:290–295.
36. Bezerra PG, Britto MC, Correia JB, Duarte Mdo C, Foncela AM, Rose K, Hopkins MJ, Cuevas LE, McNamara PS. Viral and atypical bacterial detection in acute respiratory infection in children under five years. *PLoS ONE* 2011;6:e18928. Available from: DOI: 10.1371/journal.pone.0018928
37. Garcia-Garcia ML, Calvo C, Perez-Brena P, De Cea JM, Acosta B, Casas I. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. *Pediatr Pulmonol* 2006;41:863–871.
38. Flamant C, Hallalé F, Nolent P, Chevalier JY, Renolleau S. Severe respiratory syncytial virus bronchiolitis in children: from short mechanical ventilation to extracorporeal membrane oxygenation. *Eur J Pediatr* 2005;164:93–98.
39. Welliver RC, Checchia PA, Bauman JH, Fernandes AW, Mahadevia PJ, Hall CB. Fatality rates in published reports of RSV hospitalizations among high-risk and otherwise healthy children. *Curr Med Res Opin* 2010;26:2175–2181.
40. Papoff P, Moretti C, Cangiano G, Bonci E, Roggini M, Pierangeli A, Scagnolari C, Antonelli G, Midulla F. Incidence and predisposing factors for severe disease in previously healthy term infants experiencing their first episode of bronchiolitis. *Acta Paediatr* 2011;100:e17–e23. Available from: DOI: 10.1111/j.1651-2227.2011.02181.x
41. Christensen A, Nordbo SA, Krokstad S, Rognlien AG, Dollner H. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J Clin Virol* 2010;49:158–162.