

## Viral etiology in infants hospitalized for acute bronchiolitis

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Acute bronchiolitis is predominantly a viral disease. Respiratory syncytial virus is the most common agent, but other newly identified viruses have also been considered as causes. The aim of the present study is to determine the respiratory viruses causing acute bronchiolitis in hospitalized infants. Infants younger than 2 years of age who were hospitalized for acute viral bronchiolitis in a children's hospital between November 2011 and May 2012 were evaluated for the presence of viruses as etiologic agents using a real-time polymerase chain reaction method.

A total of 55 infants were included in this study. The mean age of the children was 6.98±5.53 months, and 63.6% were male. In the 55 children, 63 viruses were detected. A single viral pathogen was detected in 47 (85.5%) patients, and two viruses were co-detected in 8 (14.6%) patients. Respiratory syncytial virus was the most common virus identified, accounting for 25 (45.5%) cases, followed by rhinovirus (n=9, 16.4%), and human metapneumovirus (n = 8, 14.5%).

Although respiratory syncytial virus remains the major viral pathogen in infants hospitalized for acute bronchiolitis, more than half of bronchiolitis cases are associated with other respiratory viruses.

**Key words:** acute bronchiolitis, human metapneumovirus, infant, polymerase chain reaction, rhinovirus, respiratory virus, respiratory syncytial virus.

Bronchiolitis is one of the most common infectious respiratory conditions of early childhood and the leading cause of hospitalization for infants. Acute bronchiolitis is characterized by tachypnea, wheezing, suprasternal or intercostal retraction, and sometimes cyanosis or apnea, or even respiratory failure<sup>1</sup>. Risk factors for severe bronchiolitis include prematurity, cardiovascular disease, chronic pulmonary disease and immunodeficiency<sup>1</sup>.

Bronchiolitis is predominantly a viral disease, with the most common acute pathogen being respiratory syncytial virus (RSV), which causes up to 75% of cases<sup>1,2</sup>. Other viruses, such as influenza A and B, parainfluenza (PIV) 1, 2, and 3, and adenovirus (ADV) have also been considered the "usual suspects" for respiratory infection. In the past decade, new viruses

associated with respiratory infection have been identified, including human metapneumovirus (hMPV), coronavirus, PIV4 and human bocavirus (hBoV). Moreover, coinfection with two or more pathogens is common<sup>3</sup>. Bronchiolitis caused by other viruses is usually clinically indistinguishable from RSV-induced disease.

A better understanding of the spectrum of respiratory viruses causing respiratory infections in hospitalized children is essential for improving therapeutic strategies and decreasing the diagnostic efforts necessary. Respiratory viruses are detected by cell cultures, serology, antigen detection and nucleic acid-based molecular methods. Polymerase chain reaction (PCR) is a very sensitive nucleic acid-based molecular method. Rapid viral tests are cost effective because of reduced hospital stay,

reduced use of antibiotics therapy and reduced use of investigational tests<sup>1</sup>.

In our country, there have been only limited studies concerning viral etiology and clinical manifestations in patients with acute bronchiolitis. The aim of the study is to determine the respiratory viruses present in infants hospitalized for bronchiolitis.

## Material and Methods

### Patients

The study population included infants who were hospitalized for acute viral bronchiolitis at Ankara Children's Hematology Oncology Training and Research Hospital between November 2011 and May 2012. Bronchiolitis is defined as a constellation of clinical symptoms and signs including a viral upper respiratory prodrome followed by increased respiratory effort and wheezing in children less than 2 years of age<sup>4</sup>.

The inclusion criteria for the study were:

Patients hospitalized for acute bronchiolitis between November 2011 and May 2012;

Both patients having first and patients having recurrent (more than one) bronchiolitis attacks;

Patients who underwent nasopharyngeal swabs and had a positive multiplex PCR result for respiratory viruses.

The exclusion criteria for study were:

Patients who had a negative multiplex PCR result;

Patients who had a lower respiratory airway infection disease other than acute bronchiolitis.

### Data Collection

We collected the demographic and clinical data retrospectively using patient medical records: date of admission, age at admission, gender, breastfeeding history, family smoking habits and number of days with respiratory symptoms. Underlying conditions (such as history of prematurity, congenital heart disease, chromosomal disorders, neuromuscular disorders, presence of other congenital abnormalities), vital signs and physical examination at admission were recorded.

Results of diagnostic tests (PCR, chest radiograph) were noted. Treatments (salbutamol, epinephrine, systemic steroids), need for

oxygen supply, length of hospital stay, pediatric intensive care unit (PICU) admission, length of stay in the PICU, need for endotracheal intubation and mortality were also recorded.

### Viral identification

In order to detect the respiratory pathogens, nasopharyngeal swabs were obtained from patients. Specimens were collected from the nasopharyngeal cavity with a special swab (sterile cotton buds), placed into a viral transport medium and delivered to the virology laboratory. Each specimen was transported to the laboratory of the Refik Saydam National Public Health Agency, where they were processed within 24 hours after collection.

Using a real-time multiplex PCR method, specimens were investigated for the presence of viral respiratory pathogens: influenza A and B, PIV types 1, 2, 3 and 4, coronavirus types 229, 63, 43 and HKU, RSV A and B, rhinovirus (RV), hMPV, hBoV, ADV, parechovirus, enterovirus and SWH1N1.

Viral nucleic acid extract was obtained from the samples using a commercial kit (total nucleic acid purification kit, Invitrogen, Carlsbad, CA, USA); virus identification was completed with an ABI7500 machine using a commercial real-time multiplex PCR kit (FTD Respiratory Pathogens, Luxembourg, Luxembourg). During PCR amplification, negative and positive controls were included in each run and an internal control included in each sample.

The study protocol was approved by the ethics committee of the hospital.

### Statistical analysis

SPSS 18.0 was used in statistical analysis. The definitions were given as number and percentage for discrete variables and mean and standard deviation for continuous variables.

## Results

### Demographic Characteristics and Risk Factors

During the study period, 197 patients with lower airway infections were hospitalized. Fifty-five patients who were diagnosed with acute bronchiolitis and who had positive PCR results were included in this study. Demographic characteristics and clinical features of the patients are shown in Table I. The mean age of the children was  $6.98 \pm 5.53$  months, ranging

from 1 to 23 months; 63.6% (n:35) were male. A total of 10 patients (18.2 %) had a previous history of prematurity, being born between 32 and 36 weeks of gestational age (GA). Birth weight ranged from 1700 to 4600 g, with 2 patients under 2000 g. Prenatal smoking was present in 7 mothers (12.7%) and parental smoking was reported for 28 infants (50.9%).

The majority (n=45, 81.8 %) of the patients were previously healthy. Ten patients had an underlying disease (3 with congenital heart disease, 3 with chromosomal disorders, 2 with metabolic disorders, 2 with neuromuscular disorders).

Mean length of the hospital stay was  $10.12 \pm 4.74$  days (min:5-max:25). Nineteen patients (34.5%) stayed in the hospital 7 days or less. Of the patients, 24 (43.6%) had a previous wheezing event before hospitalization (1 previous wheezing event in 6 patients, 3 or more wheezing events in 8 patients). Systemic steroids were administered to 44 patients (80%). Five patients (9.1%) required intensive care, and 1 patient (1.8%) needed mechanical ventilation support.

#### Microbiological Evaluations

From 55 children, 63 viruses were detected. The viruses detected are given in Figure 1. A single viral pathogen was detected in 47 patients (85.5%) and two viruses were concurrently detected in 8 patients (14.6%). RSV was the most common virus identified, accounting for 25 cases (45.5%), followed by RV (n=9, 16.4%), hMPV (n = 8, 14.5%) and hBoV (n=3, 5.4%). Three (12%) of the 25 RSV-positive children had coinfection with other viruses (2 patients with RV, 1 patient with ADV).

Five (55.5%) of the 9 RV-positive children had coinfection with other viruses (RSV in 2 patients, ADV in 1 patients, coronavirus in 1 patient, hBoV in 1 patient). None of the hMPV-positive children had coinfection with other viruses. All of the hBoV-positive children had coinfection with other viruses (1 patient with RV, 1 patient with ADV, 1 patient with influenza B).

#### Radiologic Evaluations

A chest radiograph was performed on 51 patients; 3 patients had normal radiographs. The most frequent findings were peribronchial thickening and hyperinflation.

#### Complications and Mortality

We documented 4 complications: pericardial effusion in 1 patient with RV, pneumothorax in 1 patient with hBoV, pneumomediastinum in 1 patient with RSV, and supraventricular tachycardia in 1 patient with hMPV.

We documented one mortality. The patient, who had an RSV infection, was 5 months old with underlying congenital heart disease.

#### Discussion

The present real-time PCR-based study describes the incidence and demographic and clinical characteristics of bronchiolitis caused by various viruses among infants hospitalized for acute bronchiolitis. It is the first study comprising a large PCR panel of respiratory viruses, including the novel viruses hMPV and hBoV, to be done in our country. The most common viral etiology was RSV, followed by RV and hMPV.

Acute bronchiolitis is predominantly a viral

**Table I.** Demographic Characteristics and Clinical Features of Patients

| Variable   | Number (%) |
|--|------------|
| Age (mean±standard deviation, months)              | 6.98±5.53  |
| Male gender  | 35 (63.6)  |
| History of prematurity (gestational age <37 weeks) | 10 (18.2)  |
| Maternal smoking during pregnancy                  | 7 (12.7)   |
| Parental smoking                                   | 28 (50.9)  |
| Presence of underlying chronic illness             | 10 (18.2)  |
| Hospital stay (mean±standard deviation, days)      | 10.12±4.74 |
| Use of systemic steroids                           | 44 (80.0)  |
| PICU admission                                     | 5 (9.1)    |

PICU: Pediatric intensive care unit.

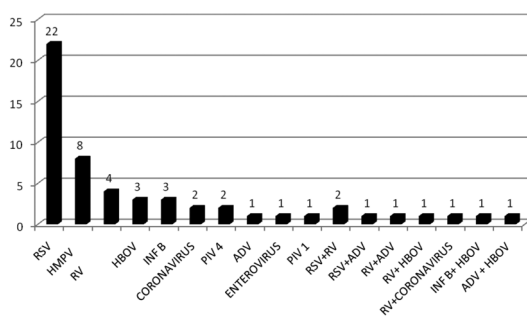


Fig. 1. Viral etiology of patients.

disease. Diagnostic tests should be able to rapidly identify viral and bacterial pathogens and thus simultaneously reduce antibiotic use<sup>3</sup>. With the development of PCR techniques, respiratory viruses, including RV, hMPV, hBoV, PIV, ADV and influenza virus, are detected rapidly. This is allowing us to better explore respiratory infections<sup>3</sup>. More data are needed to understand the exact role of the newly identified viruses. In 54.5% of our patients, viruses other than RSV were identified. After RSV, the most frequently detected viruses were RV (16.4%) and hMPV (14.5%).

The most common cause of bronchiolitis is RSV infection. The isolation rate varies according to different study designs and geographic areas. Especially in hospitalized children under 2 years of age, a frequency of RSV as high as 50-90% has been reported in acute bronchiolitis<sup>5,6</sup>. The reported frequency of RSV in hospitalized infants ranges from 20% to 63% in our country<sup>7</sup>. As in the previous literature, RSV was the most frequently detected respiratory virus in our study, accounting for 45.5% of all detected viruses.

RV has generally been considered a cause of mild upper respiratory illness in children and adults. But many recent studies have shown RV to be the second most frequent agent in acute bronchiolitis<sup>1,8</sup>. Mansbach et al.<sup>6</sup> reported a rate of 25.6% for RV, while Huguen et al.<sup>8</sup> reported a lower incidence rate (8%) in acute bronchiolitis. In our study, we detected RV as the second most common agent (16.4%). The differences between reports may have resulted from the varying design of the studies, such as including patients from different age groups and using different methods.

Another important agent causing acute bronchiolitis is hMPV. In our study, hMPV was

the third most frequent virus, after RSV and RV. In another study conducted in our country, Hatipoglu et al.<sup>9</sup> also reported that hMPV was the third most common agent. Studies have shown that hMPV infections seem to have a severity similar to that of RSV infection<sup>10-14</sup>. hMPV infections have been reported to occur in late winter to early spring, coincident with or slightly later than RSV<sup>11</sup>. Although Garcia-Garcia et al.<sup>11</sup> showed coinfections to be frequent in hMPV, none of the patients in our study had coinfections.

The incidence of hBoV was reported as between 7.1% and 27% of patients hospitalized for bronchiolitis in previous studies<sup>5,8,15</sup>. In our study, hBoV was detected in 5.4% of patients.

Multiplex PCR has been used to detect concurrent multiple respiratory virus infections. Coinfection with more than one respiratory virus is common in children (13-67%)<sup>8,16,17</sup>. However, the clinical implications and effect upon disease severity of multiple viral infections remain controversial<sup>16</sup>. In our study, 12% of RSV-infected patients and 55.5% of RV-infected patients were also infected with other viruses. Calvo et al.<sup>18</sup> reported a coinfection rate for RV (40%) that was similar to our results.

A mortality rate of 3.2% was reported in the literature, recorded in patients with severe comorbidities<sup>1</sup>. There was one mortality in our study. The patient had RSV infection and congenital heart disease.

The major limitations of our study were that only hospitalized patients were included and the study was conducted in only one season. Larger studies conducted among patients with a wider range of disease severity and taking place throughout the year may give more informative data.

In conclusion, in view of the limited number of PCR-based studies on viral respiratory tract infections heretofore conducted in Turkey, the present study depicts viral epidemiology in hospitalized infants with a focus on novel respiratory viruses. RSV is the most common agent seen as a single pathogen, followed by hMPV. Although RSV remains the major viral pathogen in bronchiolitis, more than half of bronchiolitis cases are associated with other respiratory viruses.

## REFERENCES

1. Wainwright C. Acute viral bronchiolitis in children—a very common condition with few therapeutic options. *Paediatr Respir Rev* 2010; 11: 39-45.
2. Miller EK, Gebretsadik T, Carroll KN, et al. Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. *Pediatr Infect Dis J* 2013; 32: 950-955.
3. Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. *Clin Infect Dis* 2011; 52: S284-S289.
4. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics* 2006; 118: 1774-1793.
5. Midulla F, Scagnolari C, Bonci E, et al. Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. *Arch Dis Child* 2010; 95: 35-41.
6. 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: 700-706.
7. Hacımustafoğlu M, Çelebi S, Bozdemir SE, et al. RSV frequency in children below 2 years hospitalized for lower respiratory tract infections. *Turk J Pediatr* 2013; 55: 130-139.
8. Huguenin A, Moutte L, Renois F, et al. Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. *J Med Virol* 2012; 84: 979-985.
9. Hatipoğlu N, Somer A, Badur S, et al. Viral etiology in hospitalized children with acute lower respiratory tract infection. *Turk J Pediatr* 2011; 53: 508-516.
10. Papenburg J, Boivin G. The distinguishing features of human metapneumovirus and respiratory syncytial virus. *Rev Med Virol* 2010; 20: 245-260.
11. García-García ML, Calvo C, Martín F, Pérez-Breña P, Acosta B, Casas I. Human metapneumovirus infections in hospitalised infants in Spain. *Arch Dis Child* 2006; 91: 290-295.
12. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008; 167: 63-69.
13. Kim CK, Choi J, Callaway Z, et al. Clinical and epidemiological comparison of human metapneumovirus and respiratory syncytial virus in Seoul, Korea, 2003-2008. *J Korean Med Sci* 2010; 25: 342-347.
14. Paget SP, Andresen DN, Kesson AM, Egan JR. Comparison of human metapneumovirus and respiratory syncytial virus in children admitted to a paediatric intensive care unit. *J Paediatr Child Health* 2011; 47: 737-741.
15. 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: 883-887.
16. Stempel HE, Martin ET, Kuypers J, Englund JA, Zerr DM. Multiple viral respiratory pathogens in children with bronchiolitis. *Acta Paediatr* 2009; 98: 123-126.
17. Antunes H, Rodrigues H, Silva N, et al. Etiology of bronchiolitis in a hospitalized pediatric population: prospective multicenter study. *J Clin Virol* 2010; 48: 134-136.
18. Calvo C, García-García ML, Blanco C, Pozo F, Flecha IC, Pérez-Breña P. Role of rhinovirus in hospitalized infants with respiratory tract infections in Spain. *Pediatr Infect Dis J* 2007; 26: 904-908.