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Rhinovirus Species in Children with Severe Bronchiolitis: Multicenter Cohort Studies in the US and Finland

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Abstract

In this analysis of two prospective multi-center, multi-year cohorts of children hospitalized for bronchiolitis in the US and Finland, 306 rhinovirus infections were genotyped. Rhinovirus-A and -C species were predominant in the US study while rhinovirus-C species was predominant in the Finland study. In both cohorts, there were no significant between-species differences in clinical characteristics, including acute severity measures.

Keywords

rhinovirus; species; genotypes; bronchiolitis; children; cohort study

BACKGROUND

Bronchiolitis is an important public health problem in the US and worldwide.¹ In the US, bronchiolitis is the leading cause of hospitalizations in infants, accounting for approximately 130,000 hospitalizations each year.² In addition to the large acute disease burden, cohort studies have also demonstrated substantial chronic morbidity, with 30%–40% of children with severe bronchiolitis (bronchiolitis requiring hospitalization) developing childhood asthma.¹

After respiratory syncytial virus (RSV), rhinovirus is the second most common pathogen in severe bronchiolitis, accounting for 20%–40% of cases.¹ Rhinoviruses are non-enveloped

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positive-strand RNA viruses in the family Picornaviridae, consisting of approximately 170 distinct genotypes that are classified into three species (A, B, and C).³ Epidemiological studies have reported rhinoviruses-A and -C to be the more common species in children with acute respiratory infections and wheezing illnesses.³ Despite the public health and research significance of severe bronchiolitis, little is known about the prevalence of different rhinovirus species in this inpatient population. The rhinovirus subtype literature in children has also shown conflicting data, with the acute severity found to be no different^{4,5} or greater^{6,7} with particular species.

To address this knowledge gap, we investigated the patterns of rhinovirus species in children with severe bronchiolitis and their relations to the patient characteristics – including acute severity of bronchiolitis – by analyzing the data from two prospective studies of children hospitalized with bronchiolitis in the US and Finland.

METHODS

The present analysis combines the data from two multi-center, multi-year prospective cohort studies of children with severe bronchiolitis. Using a similar protocol, one study was from the US, while the other was from Finland. The details of study design, setting, participants, and methods of data collection may be found in the **Supplemental Digital Content**. Briefly, we conducted the US study, called the 35th Multicenter Airway Research Collaboration (MARC-35), at 17 sites across 14 US states.⁸ Using a standardized protocol, investigators enrolled infants (age <1 year) hospitalized with an attending physician diagnosis of bronchiolitis during three consecutive bronchiolitis seasons (from November 1 through April 30) during 2011–2014. Bronchiolitis was defined by the American Academy of Pediatrics guidelines – acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions. Using a similar protocol, we conducted the Finland study (MARC-30 Finland) at three sites during the 2008–2010 bronchiolitis seasons.⁹ We enrolled children aged <2 years hospitalized with bronchiolitis. The institutional review board at each of the participating hospitals approved the study. Written informed consent was obtained from the parent or guardian.

In addition to the clinical data measurement through structured interview and medical record review, nasopharyngeal aspirates were collected by trained investigators using a standardized protocol within 24 hours of hospitalization in both cohorts.^{8,9} Singleplex real-time PCR was used to detect rhinovirus at Baylor College of Medicine (Houston, TX, USA). Of rhinovirus-positive specimens, their species and genotypes were identified by using molecular typing assay that targets a variable fragment in 5′ untranslated region of the viral genome flanked by highly conserved motifs at University of Wisconsin (Madison, WI, USA).¹⁰ Patients were categorized into three rhinovirus species groups (A, B, and C) in each cohort, excluding those with coinfection of multiple species. Next, between the species groups, we compared the patient characteristics as well as clinical presentation and course, including acute severity measures (i.e., intensive care use [admission to the intensive care unit and/or use of mechanical ventilation], hospital length-of-stay), between the three species using chi-square, Fisher exact, or Kruskal-Wallis tests, as appropriate. Analyses used R version 3.4. All P-values were two-tailed, with P<0.05 considered statistically significant.

RESULTS

Of 1,016 infants with severe bronchiolitis enrolled into the MARC-35 study, singleplex PCR detected rhinovirus in 214 patients (21%). Of these, molecular typing assay genotyped 204 and identified 85 unique rhinovirus genotypes. Compared to the non-typeable samples, the genotyped samples had a higher genomic load in real-time PCR (median threshold cycle value, 38 vs. 29; $P<0.001$). After excluding 7 patients with coinfection of multiple rhinovirus species, a total of 197 patients were eligible for the current analysis. Overall, the median age of patients was 4 months (IQR 2–7 months), 32% were female, and 56% had coinfection with RSV (Figure 1A).

In the MARC-30 Finland study, 408 infants with severe bronchiolitis were enrolled. Singleplex PCR detected rhinovirus in 130 patients (32%). Of these, molecular typing assay genotyped 110 and identified 46 unique rhinovirus genotypes. After excluding one patient with coinfection of multiple rhinovirus species, a total of 109 patients were eligible for the current analysis. Overall, the median age of patients was 13 months (46 patients with age <1 year and 63 patients with age 1–1.9 years), 32% were female, and 7% had coinfection with RSV (Figure 1A).

In the both cohorts (MARC-35 [age < 1 year] and MARC-30 Finland [age <1 year and 1–1.9 years]), A and/or C species accounted for the majority of rhinovirus infection (Figure 1B). In MARC-35, 47% had rhinovirus-A (38 genotypes), 6% rhinovirus-B (8 genotypes), and 47% rhinovirus-C (39 genotypes) species (**Table E1**). In MARC-30 Finland, the overall proportions were 23% with rhinovirus-A (15 genotypes), 3% with rhinovirus-B (3 genotypes), and 74% with rhinovirus-C (28 genotypes) species (**Table E2**), with similar proportions between the age groups ($P=0.94$; Figure 1).

Looking across the three species groups, there were no significant differences in most patient characteristics and clinical course in either MARC-35 (**Table E3**) or MARC-30 Finland (**Table E4**). Indeed, other than the proportion of household siblings in MARC-35 and history of corticosteroid use in MARC-30 Finland, there was no significant difference in the baseline patient characteristics, family, medical, and environmental history, laboratory testing (e.g., RSV coinfection, specific IgE sensitization) as well as acute severity markers, such as intensive care use and hospital length-of-stay (all $P>0.05$). Likewise, there was no significant difference in the geographical location ($P=0.17$ in MARC-35 USA and $P=0.87$ in MARC-30 Finland). In contrast, there was a significant difference in the enrollment year ($P<0.001$ in both cohorts).

DISCUSSION

In this analysis based on two multi-center, multi-year prospective cohorts of children with severe bronchiolitis in the US and Finland, we successfully genotyped 306 rhinovirus infections. The rhinovirus-A and -C species co-dominated rhinovirus infection – with a high proportion of coinfection with RSV – in MARC-35, while rhinovirus-C species was predominant in MARC-30 Finland. In both cohorts, there were no significant between-

species differences in most patient characteristics and clinical course, including acute severity measures.

In agreement with our finding, the literature has shown the predominance of rhinovirus-A and -C species in lower respiratory infections and wheezing illnesses.^{3–7} In contrast, previous studies on children with acute respiratory infections have reported inconsistent relationships of rhinovirus species with acute severity of illness. For example, in an analysis of 209 infants in the COAST cohort study, infections with rhinovirus-A and -C species were associated with a higher risk of moderate-to-severe acute respiratory infection compared to those with rhinovirus-B infection.⁶ Likewise, in an analysis of single-center randomized-controlled trial (Vinku2 trial) that enrolled 84 children (aged 3–23 months) with first wheezing episode, rhinovirus-C species was associated with higher severity.⁷ In contrast, a report from the New Vaccine Surveillance Network from three US counties found that, in 339 children (aged <5 years) hospitalized for acute respiratory infection, there are no between-species differences in severity of illness.⁴ Similarly, in an analysis of community-based study (the Tennessee Children's Respiratory Initiative), there were no differences in acute severity in 162 infants with rhinovirus infection.⁵ These apparent disparities may be attributable to the differences in study populations (e.g., different age criterion), designs (e.g., surveillance vs. inception cohort), settings (e.g., outpatient vs. inpatient only), analytic techniques (e.g., rhinovirus genotyping), and outcome definitions (e.g., hospitalization, intensive care use). Regardless, our study builds on these earlier reports and extends them by investigating, for the first time, the patterns of rhinovirus species and their relations to clinical characteristics exclusively in young children with severe bronchiolitis.

We also observed the difference in the rate of RSV coinfection between the two cohorts. However, the reasons for the differences are unclear. It is unlikely that the difference is fully attributable to the study design because both studies were conducted in the same fall/winter seasons with the use of a standardized protocol and identical PCR testing for all samples. Additionally, even with the use of same age criteria (age <1 year), the rate of coinfection remained relatively low in MARC-30 Finland. While speculative, the observed difference might be attributable to circulating RSV genotypes – with potentially different virulence profiles – between the two countries and multiple study years (2011–2014 in MARC-35 USA vs. 2008–2010 in MARC-30 Finland).

Our study has several potential limitations. First, bronchiolitis is a clinical syndrome without a common international definition. Despite the use of standardized protocols, it remains possible that enrolled children had different respiratory disorders. Second, our study samples consisted of children hospitalized with bronchiolitis in academic centers. Thus, the spectrum of severity in this population is relatively narrow (e.g., from hospital short-stay [<24 hours] to intubation with mechanical ventilation use). Additionally, our observations may not be generalizable to children with mild-to-moderate bronchiolitis in other settings. Regardless, our data are directly relevant to hundreds of thousands of children with severe bronchiolitis each year.² Lastly, the current study design precluded us from examining the relationship between rhinovirus species in bronchiolitis and *chronic* respiratory outcomes (e.g., incident asthma). To address this important question, both study cohorts are currently being followed longitudinally.

In summary, on the basis of two multi-center, multi-year prospective studies of children hospitalized for bronchiolitis in the US and Finland, we found that rhinovirus-A and/or -C are the predominant species. Within in this population, we also observed that there were no significant between-species differences in most patient characteristics and clinical course, including acute severity measures. For clinicians, evidence to predict the acute disease course in children with severe bronchiolitis remains limited.¹ However, our data should facilitate further investigations into the link between bronchiolitis with different rhinovirus species and the development of childhood asthma.

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Figure 1. Proportions of coinfection with respiratory syncytial virus (RSV) and rhinovirus species in two cohort studies of children with severe bronchiolitis

Because of the difference in the inclusion criterion of age (age <1 year in MARC-35 and age <2 years in MARC-30 Finland), the MARC-30 Finland cohort was grouped into two groups by age (age <1 and 1–1.9 years).

A) The proportions of RSV coinfection differed significantly between the three cohort groups ($P<0.001$), while the proportions did not differ significantly within the MARC-30 Finland cohort ($P=0.07$).

B) The proportions of rhinovirus species differed significantly between the three cohort groups ($P=0.003$), while the proportions did not differ significantly within the MARC-30 Finland cohort ($P=0.94$).