Comparison of the Severity of Respiratory Disease in Children Testing Positive for Enterovirus D68 and Human Rhinovirus

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Objective To compare the characteristics and severity of respiratory disease in children testing positive for enterovirus D68 (EV-D68) and for human rhinovirus (RhV).

Study design A retrospective single center study of children presenting with acute respiratory symptoms and positive polymerase chain reaction for RhV/EV from September 1, 2014 through October 31, 2014 was performed. Specimens were subsequently tested specifically for EV-D68 and specimens identified as RhV were subtyped when possible into RhV-A, RhV-B, and RhV-C species. Clinical manifestations in patients with EV-D68 were compared with those with non-EV-D68, RhV, and RhV-C.

Results Of the 173 patients included in the analysis, 72 tested positive for EV-D68, 61 for RhV, and 30 for RhV-C. There were significantly fewer infants in the EV-D68 group. Patients with EV-D68 were more likely than those without EV-D68, and specifically with RhV-C, to have fever and wheezing. Patients with EV-D68 received more magnesium sulfate for respiratory distress not responding adequately to repeated doses of inhaled albuterol. Hospitalized patients with EV-D68 received more bronchodilator therapy than patients with RhV. Patients with EV-D68 were more likely to be admitted to the intensive care unit and were older than patients without EV-D68. There was no difference in length of overall hospitalization or time in the pediatric intensive care unit.

Conclusions Children with EV-D68 appeared to have more severe respiratory disease on admission than children with RhV as evidenced by higher rates of fever, wheezing, bronchodilator use and pediatric intensive care unit admission. Despite the initial difference in severity, no significant difference in length of stay was found suggesting that patients with EV-D68 recovered as quickly as other groups. *(J Pediatr 2018;■■:■■-■■).*

From August through December 2014, a nationwide outbreak of severe respiratory disease due to enterovirus D68 (EV-D68) occurred in children throughout the US. As of January 15, 2015, the Centers for Disease Control and Prevention and state public health laboratories confirmed a total of 1153 people in 49 states and the District of Columbia with respiratory illness caused by this previously rarely reported virus. Nearly all reported cases were in children, many of whom had a history of asthma or wheezing.[1] Increased incidence of EV-D68 was found in other parts of the world as well, including Canada, Central America, Europe, and Asia.[2-8]

EV-D68 was first isolated in 1962 from 4 children in California with bronchiolitis and pneumonia,[9] with infrequent reports of disease due to EV-D68 until the outbreak in 2014.[10,11] Although classified as an enterovirus, EV-D68 has been described as producing respiratory symptoms, similar to other rhinovirus species within the genus enterovirus in the family *Picornaviridae*. Shared properties between EV-D68 and rhinoviruses include sensitivity to acidic environments and preference for lower temperatures, making the nasal and respiratory mucosa more hospitable environments than the gastrointestinal tract, often preferred by other types of enterovirus.[12] Human rhinoviruses (RhV) are the most common cause of upper and lower respiratory tract infections in humans and are divided into 3 separate species: RhV-A, RhV-B, and RhV-C. RhVs are a frequent viral cause of wheezing in infants and young children and commonly trigger asthma exacerbations in both adults and children.[13-15] Several studies have associated RhV-C with more severe respiratory illness and wheezing.[16-19] Until the 2014 outbreak, only small outbreaks of EV-D68 respiratory illness had been described, with limited clinical information. Publications from the 2014 outbreak primarily have compared patients with EV-D68 with patients who tested positive by multiplex polymerase chain reaction

EV-D68	Enterovirus D68
LOS	Length of stay
MFCH	Maria Fareri Children's Hospital
NP	Nasopharyngeal
NYSD	New York State Department of Health
PCR	Polymerase chain reaction
RhV	Human rhinovirus
RhV/EV	Human rhinovirus/enterovirus
rRT-PCR	Reverse transcription real-time PCR
WMC	Westchester Medical Center

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(PCR) assay for human rhinovirus/enterovirus (RhV/EV) but in whom specific EV-D68 PCR testing was negative. These studies did not further delineate which of the specimens negative for EV-D68 were positive for RhV, and did not include RhV subtyping.

The objectives of this study are to describe the epidemiology of patients with EV-D68 presenting to a single children's hospital during the nationwide outbreak, and to compare disease characteristics and risk factors in children with EV-D68-associated respiratory disease with non-EV-D68 virus-associated respiratory disease, including data for confirmed RhV and RhV species.

Methods

A retrospective chart review was performed to compare the risk factors, disease characteristics, and management of respiratory disease in children testing positive for EV-D68 vs non-EV-D68 rhinoviruses and enteroviruses during the EV-D68 outbreak in 2014. The study was approved by the New York Medical College Institutional Review Board. Charts were reviewed for all patients with positive nasopharyngeal (NP) swab RhV/EV testing by the Biofire Respiratory Panel multiplex PCR assay (Version 1.7, BioFire Inc, Salt Lake City, Utah) who presented to the Maria Fareri Children's Hospital (MFCH) at Westchester Medical Center (WMC) from September 1 to October 31, 2014. Patients were eligible for inclusion in the analysis if they presented with any upper or lower tract respiratory symptoms. At our institution the Respiratory Panel multiplex assay typically is performed on patients presenting with moderate to severe respiratory symptoms. Patients seeking care before September 1, 2014 or for nonrespiratory complaints were excluded. Patients testing positive for RhV/EV plus a second virus were excluded. For patients hospitalized more than once during the study period, only data from the first admission is included. Demographic data, dates of admission, testing, and discharge were electronically abstracted, and clinical data were manually abstracted.

The number of positive RhV/EV tests by week was provided by the WMC Microbiology Laboratory for 2013 and 2014 to compare rates over 2 years. The Biofire Respiratory Panel multiplex PCR assay was implemented at WMC in January 2013, no RhV/EV testing was available before that date.

Virology Methods

All respiratory specimens from pediatric patients that tested positive for RhV/EV by the Biofire Respiratory Panel multiplex PCR assay during the study period, when sufficient sample remained, were tested for EV-D68 by a reverse transcription real-time PCR (rRT-PCR) assay as previously described.[20] Confirmation of rRT-PCR results and RhV species determination was performed by Sanger sequencing, in which partial sequences in VP-1 and 5' UTR of the EV/RhV genome were amplified. Next-generation sequencing also was performed on selected specimens as previously described.[21] Upon recognition of the outbreak at MFCH, 81 NP specimens positive for RhV/EV were sent to the New York State Department of Health (NYSDOH) Wadsworth Center Laboratory for EV-D68 testing. Seventeen of the 81 samples sent to NYSDOH were able to be retrieved and testing was performed as described above at the WMC laboratory. For patients included in our analysis, virologic results from NYSDOH were used if sample was not available for WMC testing.

Statistical Analyses

For the primary analysis, the following 2 groups were compared: patients testing positive for EV-D68 and patients testing positive for RhV/EV but negative for EV-D68 by rRT-PCR assay. Additional analyses were performed comparing subjects with EV-D68- to confirmed RhV (A, B, and C), and specifically to subjects with RhV-C. There were too few subjects in the RhV-A and RhV-B groups to perform separate analyses.

Patient characteristics were evaluated for potential risk factors including age, sex, race, ethnicity, demographic variables, and comorbid conditions. Categorical values were summarized by frequencies, and continuous variables were summarized by median and IQR. Univariable analysis was conducted to determine the association between potential risk factors and EV-D68. Categorical variables, such as age and comorbid conditions, were compared using χ^2 analysis. Age for the entire study population was further divided into quartiles, and comparisons between groups were performed using χ^2 analysis. Continuous variables, such as length of stay (LOS), were compared using the Mann-Whitney rank sum test. Stratified analysis, using Mantel-Haenszel test, was performed to evaluate the effects of each variable of interest as a possible confounder.

A multivariable analysis was conducted as well, using multiple logistic regression to evaluate the relationship between patients testing positive for EV-D68 and the outcome of PICU admission. All variables with a P value of <0.2 on univariate analysis were included in the multivariable model.

All statistical analyses were performed using SigmaPlot 13.0 software (Systat Software Inc, San Jose, California).

Results

The 2014 EV-D68 outbreak occurred during the epidemiologic weeks 35-42, which overlapped with the epidemiologic weeks in 2013 (weeks 37-42) when there were high rates of RhV/EV-positive nasal swabs in our institution (**Figure 1**; available at www.jpeds.com).

The study participant flow diagram is detailed in **Figure 2**; 540 NP swabs were sent for respiratory virus testing on 492 pediatric patients and 233 (43%) tested positive for RhV/EV. Patients with nonrespiratory symptoms (N = 45) and those with coinfections with more than1 respiratory virus were excluded (N = 15). The analysis included 173 patients: 72 (42%) were positive for EV-D68; 61 (35%) positive for RhV on VP1 sequencing; and 40 (23%) were negative for EV-D68 but could not be further speciated. The inability to determine species for 40 of the samples was primarily due to insufficient remaining sample after initial testing or poor quality PCR amplicon products that could not be sequenced. Of the 61 samples positive for RhV, RhV-C was identified most frequently with 30

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