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Is virus coinfection a predictor of severity in children with viral respiratory infections?

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Abstract

Molecular assays have resulted in increased detection of viral respiratory infections, including virus coinfection, from children with acute respiratory infections. Yet the clinical severity of virus coinfection compared to single virus infection remains uncertain. We performed a retrospective study of children presenting with acute respiratory infections comparing clinical severity of single respiratory virus infection to virus coinfection, detected on midturbinate swabs by molecular assays. Patient characteristics and measures of clinical severity were abstracted from health records. A total of 472 virus-infected children were included, 391 with a single virus infection and 81 with virus coinfection. Virus status did not affect admission to hospital (odds ratio (OR) = 0.8; 95 % confidence interval (CI) 0.5–1.4; p 0.491) or clinical disease severity among inpatients (OR = 0.8; 95% CI 0.5–1.5; p 0.515) after adjusting for age and underlying comorbidities. However, children infected with rhinovirus/enterovirus (HRV/ENT) alone were more likely to be admitted to the hospital compared to those coinfected with HRV/ENT and at least another virus, although this was not significant in multivariable analyses (OR 0.47; 95% CI 0.22–1.0; p 0.051). In multivariable analyses, children coinfected with respiratory syncytial virus (RSV) and other viruses were significantly more likely to present with radiologically confirmed pneumonia compared to those with an isolated RSV infection (OR 3.16, 95% CI 1.07–9.34, p 0.037). Equivalent clinical severity was observed between children with single virus infection and virus coinfection, although children coinfected with RSV and other viruses presented more frequently with pneumonia than those with single RSV infection. Increased disease severity observed among children with single HRV/ENT infection requires further investigation.

Keywords: Clinical disease severity, molecular diagnosis, respiratory syncytial virus (RSV), rhinovirus/enterovirus (HRV/ENT), viral respiratory infection, virus coinfection

Original Submission: 17 April 2014; **Revised Submission:** 21 July 2014; **Accepted:** 4 August 2014

Editor: L. Kaiser

Article published online: 29 October 2014

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Introduction

The recent use of molecular assays for virus detection has resulted in the identification of respiratory viruses in almost 70% of children admitted with lower respiratory tract infection, commonly defined as any patient with cough, tachypnea and/or any respiratory distress or wheezing [1]. Simultaneous detection of multiple virus pathogens has been reported in 30% of such cases [1–7]. Although respiratory syncytial virus (RSV) and influenza A (FLU-A) have been mainly identified among children with single virus infection, other viruses, including human bocavirus (HBoV), have been mainly reported in children with coinfection [1,8,9]. To date, the relationship between the clinical severity and infection status with single vs. multiple respiratory viruses remains uncertain.

http://dx.doi.org/10.1016/j.cmi.2014.08.024

The objective of this study was to compare the clinical characteristics and the severity of illness in children with multiple simultaneous respiratory virus infections to those with single virus infection while mitigating the shortcomings encountered in previous studies by (1) using a large data set including outpatients in addition to inpatients, (2) considering a composite end-point to assess the severity in patients admitted to hospital and (3) conducting multivariable analysis to adjust for potential confounding variables.

Materials and methods

Participants and definitions

We performed a single-center retrospective study of children presenting to the Hospital for Sick Children, Toronto, with an acute respiratory illness and at least one viral infection documented by molecular assays from midturbinate swabs. Specimens were collected from November 2007 to April 2008 and January to March 2009, the time periods during which multiplex polymerase chain reaction (PCR) testing was utilized in randomly selected patients under 18 years of age presenting with any respiratory symptom. Multiple simultaneous virus infections (herein referred to as coinfection) were those in which two or more virus pathogens were detected from the same respiratory sample. Virus infection status referred to virus coinfection vs. single respiratory virus infection. Information on patient demographics, relevant baseline characteristics and outcomes were extracted from health records. Underlying comorbidities were grouped into three mutually exclusive categories: cardiorespiratory condition, prematurity and any immunosuppressive/metabolic condition. In cases of multiple comorbidities, patients were assigned to the group considered to be highest-risk comorbidity, with an underlying immunocompromised/metabolic condition considered the highest-risk comorbidity, followed by a cardiorespiratory condition and then prematurity.

Bacteria coinfection was defined as the presence of any bacterial pathogen, identified by culture from blood or respiratory samples upon initial consultation with respiratory symptoms or within 30 days of their initial consultation in association with a documented viral infection. *Staphylococcus epidermidis* was considered a pathogen if isolated from more than one peripheral blood culture, from one central line blood culture or from one peripheral blood culture in high-risk patients such as those with underlying immunosuppressive conditions, prosthetic devices or newborns, according to recent guidelines. Positive bacterial urinary or stool cultures or bacteria pathogens identified from skin or wound swabs were not considered as bacteria coinfection [10].

Severity of illness was measured by two primary outcomes: hospital admission for the entire cohort, and a composite end point of intensive care (ICU) admission, hospitalization >5 days, oxygen requirements or death in hospitalized patients. Secondary outcomes examined were radiologically confirmed pneumonia, ICU admission and mortality. Ethics approval was obtained from the Research Ethics Board at The Hospital for Sick Children.

Virologic studies

From November 2007 to April 2008 and January 2009 to March 2009, 750 midturbinate flocked swabs (Copan Diagnostics, Murrieta, CA) collected in 3 mL of universal transport medium (Copan Diagnostics) were randomly selected among all specimens collected from hospitalized children with symptoms of acute respiratory tract infection. Specimens were submitted to the clinical laboratory for routine testing for respiratory viruses, which comprised direct fluorescent antigen assay (DFA) and culture. All specimens received in the laboratory were set up for routine examination, and the remaining material was immediately formed into aliquots and frozen at −80°C until further testing. The first 25 specimens received each week for a 24-week period were selected, for a total of 600 specimens from 2007 to 2008. The same method was used in 2009 to obtain a further 150 specimens. In preparation for PCR, a single aliquot of each specimen was thawed and extracted on the biorobot M48 workstation using the MagAttract Virus Mini M48 kit (Qiagen, Mississauga, ON, Canada) and eluted in 100 µL of elution buffer. The DNA obtained was divided into six aliquots and immediately frozen at −80°C until further testing. One aliquot from each specimen was subsequently tested by each of four different nucleic acid amplification-based assays: ResPlex II v2.0 (Qiagen); Seeplex RV15 kit (Seegene Inc., Seoul, Korea); xTAG-RVP and xTAG-RVP Fast Luminex, Austin, TX). These assays together detect up to 18 respiratory viruses: RSV (A, B), OC43, 229E, NL63, HKU1, rhinovirus/enterovirus (HRV/ENT), coxsackie/echovirus, parainfluenza virus (PIV) (1–4), FLU-A, FLU-B, HBoV, adenovirus (ADV) (A, B, C, D, F) and human metapneumovirus (hMPV). ResPlex II v2.0 and Seeplex RV15 assays distinguished HRV from ENT, whereas xTAG-RVP and xTAG-RVP fast assays reported a combined result of HRV/ENT as reported in our study. All specimens were also examined by DFA for 8 respiratory viruses: RSV, FLU A/FLU B, PIV 1–3, ADV (SimulFluor, Millipore, Temecula, CA) and hMPV (Diagnostic Hybrids, Athens, OH)) and/or virus culture. We defined a positive viral result as truly positive if the sample tested positive by virus culture regardless of other tests, or by DFA and at least one molecular test or by two different molecular tests. For viruses that are only detectable by molecular assays (HRV/ENT, coronaviruses, HBoV and PIV 4), a truly positive result was defined as two or more positive test results from the four molecular assays [11].

Statistical analyses

Standard descriptive and comparative statistics were used on data categorized by virus infection status (virus coinfection and single respiratory virus infection). For all statistical testing, only the first midturbinate swab was included in cases with multiple swabs within the same child because these samples cannot be considered independent. For skewed data (age), we derived medians and used the Mann-Whitney method for comparisons. The χ^2 test or Fisher's exact test was used to compare categorical variables between groups as appropriate. Multivariable logistic regression was used to assess the clinical correlates of disease severity between children infected with single respiratory viruses and those with viral coinfections in a model adjusted for age and underlying comorbidities. All available predictors perceived to be important on the basis of the literature regardless of their significance in univariable analysis were included [8]. Multivariable regression analyses were performed for each virus (HRV/ENT and RSV coinfection vs. single infection) when allowed by the sample size. A two-sided p value of <0.05 was considered to be statistically significant. Data were analysed by SPSS statistical software, version 20.0 (IBM, Armonk, NY).

Results

Patient characteristics

A total of 750 midturbinate swabs from 742 children suspected to have a respiratory illness were tested for respiratory viruses by DFA, cell culture and four molecular assays. Of these 742 children, 391 (82.8%) tested positive for a single respiratory virus and 81 (17.2%) for more than one respiratory virus. Three hundred one children (63.8%) were boys, with a median age of 1.2 years (interquartile range 0.4–3.7 years). Ninety-two children (43.8%) presented with chest x-ray–confirmed pneumonia and 264 (55.9%) with common cold. An underlying comorbidity was documented in 156 patients (33%): 80 (16.9%) had an underlying immunosuppressive condition and 36 (7.6%) had a preexisting cardiorespiratory illness. There was no difference in baseline demographic characteristics between children with single respiratory virus infection and those with virus coinfection (Table 1). Underlying cardiorespiratory conditions were overrepresented among children infected with HRV/ENT alone compared to those coinfected with HRV/ENT and at least one other virus (32.2 % vs. 8.2 %; p 0.001), but not with any other virus.

Proportion of viruses detected

Altogether, HRV/ENT (167/472; 35.4%), RSV (140/472; 29.7%), FLU (95/472; 20.1%) and coronavirus (42/472; 8.9%) were the most commonly detected viruses. FLU (88/95, 92.6%), RSV (104/140; 74.3%), HRV/ENT (118/167; 70.7%), hMPV (30/41; 73.2%) and PIV (17/32; 53.1%) were more frequently detected as single respiratory virus infections, whereas HBoV (17/24; 70.8%), ADV (9/15; 60%) and coronavirus (23/43; 53.5%) were more often identified in coinfection with other respiratory viruses. Among the 81 children with virus coinfection, only four had more than two viruses detected. In the 77 children with dual virus coinfections, HRV/ENT-RSV (13/77; 16.9%), coronavirus-RSV (12/77; 15.6%) and HRV/ENT-HBoV (9/77; 11.7%) were the most common combinations identified.

TABLE 1. Baseline characteristics of children with single virus infection and virus coinfection

Characteristic	Single infection (n = 391)	Coinfection (n = 81)	p
Age, years n (%)	1.2 (0.4–4.0)	1.2 (0.5–2.3)	0.49
Gender, male n (%)	245 (62.7)	56 (69.1)	0.31
Underlying comorbidity n (%)			
Cardiorespiratory	33 (8.4)	3 (3.7)	0.14
Prematurity	29 (7.4)	11 (13.6)	0.07
Immunocompromised/ metabolic	63 (16.1)	17 (21)	0.28

Data are presented as mean (interquartile range) or n (%).

The most common bacteria pathogen identified in blood cultures from patients with any viral infection was *Staphylococcus epidermidis* (9.3%). Of the 92 children with pneumonia, 23 (25%) presented with bacteria coinfection: 6 (6.5%) with *Staphylococcus epidermidis* bacteremia, 3 (3.3%) with *Pseudomonas aeruginosa* bacteremia, 3 (3.3%) with *Staphylococcus aureus* isolated from bronchoalveolar lavage (BAL) samples and 4 (4.3%) with *Haemophilus influenzae* cultured from BAL samples. The overall rate of bacteria coinfection documented with a virus infection was low. Similar rates of bacteria coinfection were observed between patients with single virus infection vs. those coinfected with at least another virus (12 % vs. 8.6%; p 0.662).

Clinical outcomes

In univariable analysis, children with any virus coinfection were significantly more likely to present with pneumonia compared to those with any single respiratory virus infection (29.6% vs. 17.4%; p 0.048), although this was not statistically significant by multivariable analysis (odds ratio (OR) 1.7; 95% confidence interval (CI) 0.9–3.1; p 0.102). However, children coinfected with RSV and at least another virus were significantly more likely to present with pneumonia compared to those with a single RSV infection (OR 3.16, 95% CI 1.1–9.3; p 0.037) in multivariable analyses. Eight children with single virus infection died. Among these, 5 presented with HRV/ENT infection, one with progressive acute respiratory distress syndrome after ADV infection and died 4 weeks after FLU-A (H1N1) pdm09 (pH1N1) infection, one with HBoV infection and one with PIV 4

infection. All but one fatal case had underlying cardiorespiratory or immunosuppressive/metabolic conditions. Of the five fatalities with HRV/ENT infection, three had underlying immunosuppressive conditions and two had a cardiac condition. Two of these patients died from a sepsislike picture with respiratory failure and possible underlying pneumonia with no other pathogens identified, thus suggesting that HRV/ENT may have potentially contributed to mortality. The remaining three patients likely died of their underlying disease. No deaths were observed among children with virus coinfection (Table 2).

Predictors of hospitalization

In multivariable analysis, virus status did not affect admission to hospital after adjustment for age and the presence of underlying conditions. However, when analysed by virus pathogen, children infected with HRV/ENT alone were more likely to be admitted to the hospital (73.3% vs. 49%; p <0.004) compared to those coinfected with HRV/ENT and at least another virus, although this was not statistically significant after adjusting for age and underlying comorbidities (OR 0.5; CI 0.2–1.0; p 0.051). Underlying cardiorespiratory conditions, immunodeficiency or metabolic diseases, prematurity and age were significant predictors of hospital admission in multivariable analysis (Table 3).

Predictors of clinical disease severity among inpatients

In multivariable analysis, virus status did not affect the clinical severity of disease in those admitted to the hospital. When analysed by viral pathogen, children admitted to the hospital for HRV/ENT alone were more likely to present with severe clinical disease (50% vs. 30.6%; p <0.026) compared to those coinfected with HRV/ENT and at least another virus, although this was not statistically significant in multivariable analyses (OR 0.51; 0.25–1.04; p 0.064). Underlying cardiorespiratory, immunodeficiency/metabolic diseases and prematurity remained significant predictors of clinical disease severity among inpatients in multivariable analysis (Table 4).

TABLE 2. Clinical outcomes of children with single virus infection and virus coinfection

Characteristic	Univariable analysis: Single infection (n = 391)	Univariable analysis: Coinfection (n = 81)	p	Multivariable analysis, OR (95% CI)[a]
Pneumonia	68 (17.4%)	24 (29.6%)	0.048	1.7 (0.9–3.1)
Hospital admission	214 (54.7%)	41 (50.6%)	0.499	0.9 (0.6–1.5)
ICU admission	65 (16.6%)	11 (13.6%)	0.498	0.9 (0.4–1.8)
Mortality	8 (2.1%)	0	0.168	—[b]

OR, odds ratio; CI, confidence interval; ICU, intensive care unit.
[a]Multivariable analysis was adjusted for age and the underlying comorbidities (cardiorespiratory, immunocompromised/metabolic, prematurity).
[b]Data not provided due to insufficient number of cells.

TABLE 3. Predictors of hospitalization in 255 children

Predictor	Univariable analysis: OR (95% CI)	p	Multivariable analysis[a]: OR (95% CI)
Age	1.1 (1.1–1.2)	<0.001	1.1 (1.0–1.2)
Single virus infection vs. coinfection	0.9 (0.5–1.4)	0.499	0.8 (0.5–1.4)
Underlying comorbidity		<0.001	
None	Reference		Reference
Cardiorespiratory	3.9 (1.7–9.0)	0.002	3.8 (1.6–8.9)
Prematurity	1.9 (0.9–3.7)	0.078	2.7 (1.4–5.6)
Immunocompromised/metabolic	4.6 (2.5–8.4)	<0.001	4.5 (2.5–8.4)

OR, odds ratio; CI, confidence interval.
[a]Multivariable analysis was adjusted for age and underlying comorbidities (cardiorespiratory, immunocompromised/metabolic, prematurity).

Discussion

Three important observations made in our study are: (1) no differences in clinical severity were observed between children with virus coinfection compared to those with single virus infection; (2) children with single HRV/ENT infection had more severe disease compared to those coinfected with HRV/ENT and at least another virus; and (3) children coinfected with RSV and at least another virus presented more frequently with radiologically confirmed pneumonia compared to those with single RSV infection.

Our rate of coinfection (17.2%) was similar to those of other studies [12–14] but higher than that reported by one study [15]. Discrepancy in rates of coinfection ranging from 12% to 50% [12–15] may result from the population studied (infants vs. older children; differing proportions and types of underlying comorbidities) as an underlying respiratory conditions (asthma, cystic fibrosis) may result in higher rates of coinfection. As in some studies [12–14,16,17], HRV/ENT and RSV were the most

TABLE 4. Predictors of severity as measured by composite end point of admission to ICU, hospitalization >5 days, oxygen requirements or death

Characteristic	Univariable analysis: OR (95% CI)	p	Multivariable analysis: OR (95% CI)
Age	1.1 (1.0–1.1)	0.011	1.1 (1.0–1.1)
Single virus infection vs. coinfection	0.9 (0.5–1.4)	0.555	0.8 (0.5–1.5)
Underlying comorbidity		<0.001	
None	Reference		Reference
Cardiorespiratory	3.9 (1.9–7.9)	<0.001	3.9 (1.9–8.1)
Prematurity	1.7 (0.9–3.2)	<0.001	1.5 (1.2–4.9)
Immunocompromised/metabolic	3.3 (2.0–5.5)	<0.001	3.5 (2.1–5.8)

There were 159 children with severe disease among those hospitalized.
ICU, intensive care unit; OR, odds ratio; CI, confidence interval.

common pathogens identified in our cohort, mostly as single virus infections. In contrast, two studies [12,13], which focused on severe acute respiratory illness in children in South Africa, reported HRV/ENT and RSV most frequently in coinfection with at least another virus, suggesting that the population studied (developing vs. developed countries; infants vs. older children) and the proportion of children with severe respiratory illness may influence the rates of documented coinfection.

We report no association between virus status and various measures of clinical disease severity. Studies focusing on the severity of virus coinfection have resulted in divergent findings. Possible explanations include variation in age groups, breadth of illness severity, the proportion with underlying conditions and their adjustment in multivariable analyses. When analysed by virus pathogen, we reported higher rates of hospitalization and increased clinical disease severity among hospitalized children with HRV/ENT infection alone compared to those coinfected with HRV/ENT and at least another virus, although this was not statistically significant in multivariable analysis. However, our small sample size of HRV/ENT-positive patients may have limited our findings in multivariable analyses, thus still suggesting that HRV/ENT-positive status may remain an independent predictor for severity. Underlying cardiorespiratory conditions, immunodeficiency and metabolic disorders, which were over-represented among children with single virus infection, remained the most important predictors for severe disease as reported in our multivariable analyses. As suggested in other studies [18,19], subjects coinfected with RSV and at least another virus (especially RSV-HBoV) in our cohort had higher rates of pneumonia compared to those with RSV infection alone. Possible explanations include increased inflammatory markers induced by the presence of multiple viruses thus favouring progression to pneumonia. Given our low rates of bacteria coinfection, their contribution to the development of lower respiratory tract infection was minor.

An important strength of our study was the use of 4 different molecular assays for the detection of most of the known viral respiratory pathogens, including HRV/ENT and HBoV. The impact of underlying comorbidities on clinical severity was addressed by multivariable analysis. Finally, our study enabled more extensive comparison groups (single vs. coinfection HRV/ENT, RSV) each with adequate sample sizes, although our sample size did not allow subgroup analyses for all individual viruses, influenza included. Given our low rate of detected bacteria coinfection, no adjustment for this variable was done in multivariable analyses. Potential limitations of our study relate to its retrospective design, which may have led to selection bias, as not all consecutive patients were tested for respiratory viruses. However, the random selection of the respiratory samples tested by molecular assays reduced the risk of selection bias. Second, we assessed presence or absence of single infection or virus coinfection, but we did not measure their virus load, which might have helped elucidate the association between HRV/ENT and severe disease, as rhinoviruses can be detected in up to 25% of asymptomatic patients [1,3,16]. However, inconsistency of the matrix between individuals may limit comparison of virus loads between patients. Finally, our estimation of bacteria coinfection may have been underestimated, as BAL are rarely performed in children and pneumonia rarely results in positive blood cultures. This limitation inherent to studies assessing the severity of respiratory illnesses in children may be overcome in future studies, which may incorporate novel diagnostic approaches such as nuclear magnetic resonance–based metabolomics analysis of urine for the diagnosis of bacterial pneumonia [20].

In conclusion, our findings support equivalent disease severity between single virus infection and virus coinfection and provide new insight into the impact of non-RSV respiratory coinfection on the severity of RSV and potential increased severity of single HRV/ENT infections. Future studies should be adequately powered to allow extensive virus subgroup analysis, as severity between single virus infection and virus coinfection may differ by virus pathogen.

Transparency declaration

In-kind contribution of ResPlex II v2.0 kits was provided by Qiagen. SR received in-kind support from Qiagen and Luminex Molecular diagnostics for a study of multiplex respiratory PCR in children. The funding agencies had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. All authors report no conflicts of interest relevant to this article.

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