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Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection

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Abstract

Objective: To compare the severity of single respiratory syncytial virus (RSV) infections with that of coinfections.

Methods: A historical cohort was studied, including hospitalized infants with acute RSV infection. Nasopharyngeal aspirate samples were collected from all patients to detect eight respiratory viruses using molecular biology techniques. The following outcomes were analyzed: duration of hospitalization and of oxygen therapy, intensive care unit admission and need of mechanical ventilation. Results were adjusted for confounding factors (prematurity, age and breastfeeding).

Results: A hundred and seventy six infants with bronchiolitis and/or pneumonia were included in the study. Their median age was 4.5 months. A hundred and twenty one had single RSV infection and 55 had coinfections (24 RSV + adenovirus, 16 RSV + human metapneumovirus and 15 other less frequent viral associations). The four severity outcomes under study were similar in the group with single RSV infection and in the coinfection groups, independently of what virus was associated with RSV.

Conclusion: Virus coinfections do not seem to affect the prognosis of hospitalized infants with acute RSV infection.

J Pediatr (Rio J). 2011;87(4):307-313: Coinfections, respiratory viruses, infants.

Introduction

Most infections of the lower respiratory tract in infants are caused by viruses. Respiratory syncytial virus (RSV) infections are the major cause of hospitalization among infants and are responsible for at least 3.4 million hospital admissions of children younger than 5 years all over the world.[1] Other viruses are also important etiological factors of respiratory infections in infancy: human metapneumovirus (hMPV); adenovirus (ADV); parainfluenza (PIV) 1, 2 and 3; influenza (Flu) A and B; rhinovirus; bocavirus; and coronavirus.[2-4] Viral coinfections gained greater attention

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after the introduction of molecular biology techniques, such as polymerase chain reaction (PCR), which can detect not only a larger number of viruses, but also more than one virus using the same respiratory secretion specimen. These techniques have been used to show the variable prevalence of coinfections by respiratory viruses. In children hospitalized due to severe bronchiolitis, coinfection may reach 70% according to some reports, although most studies show that prevalence rates range from 15 to 39%.[2-5]

Presently, the importance of detecting multiple viral agents in respiratory secretions remains unclear. In clinical practice, the presence of more than one viral agent generates uncertainties about the prognosis of infections. Some authors reported similar clinical progression for coinfections and infections by a single virus, whereas others suggested that, in infants with bronchiolitis, coinfection may increase the severity of the disease. This controversy became even greater after studies found lower severity rates in cases of coinfection.[5-7]

In this study, severity of RSV coinfections with other viruses is compared with severity of infections caused by a single pathogen in infants hospitalized due to acute lower respiratory tract disease. Molecular biology techniques were used to detect 8 respiratory tract viruses.

Patients and methods

This study included a historical cohort of infants with acute lower respiratory tract infections admitted to the Pediatric Clinic Division of the University Hospital of Universidade de São Paulo from February to November 2005. This hospital provides secondary care to a population of about 400,000 people in the western region of the city of São Paulo, Brazil. Inclusion criteria were: (a) age below 2 years; (b) respiratory symptoms for up to 7 days at admission, characterized by tachypnea and adventitious lung sounds at physical examination; and (c) positive detection of RSV in nasopharyngeal aspirate (NPA) collected in the first 5 hospitalization days.

First, all infants that underwent NAP collection for viral testing were selected. Patients were excluded if they had diagnoses of other associated morbidities or infection by bacteria, fungi or any other microorganism other than respiratory viruses. Patients were also excluded if they had other hospitalizations in the previous 30 days (Figure 1).

Data were collected from patient charts by one of the authors according to standardized protocols. The variables analyzed were demographic characteristics (age and sex), signs and symptoms at admission, discharge diagnosis, prematurity (gestational age < 37 weeks), bronchopulmonary dysplasia, heart disease, immunosuppression and neuropathy. Severity was assessed according to the following outcomes: total hospitalization time; oxygen therapy duration, admission to intensive care unit (ICU) and mechanical ventilation. In the service where this study was conducted, oxygen therapy is prescribed to keep oxygen saturation above 92% according to pulse oximetry. Criteria for ICU admission were clinical and laboratory signs of respiratory insufficiency that indicated the imminent need of mechanical ventilation and maintenance of oxygen saturation levels below 92% in patients receiving an inspired fraction of oxygen greater than or equal to 60%.

During the study, NPA of infants with respiratory problems was routinely collected for viral testing. Because of the hours of the Virology Laboratory of the Institute of Biomedical Sciences of Universidade de São Paulo, patients admitted between Sunday and Friday (up to 5 pm) had NPA collected on the first hospitalization day and sent to the virology laboratory on the same day. The material from patients hospitalized after 5 pm on Fridays, Saturdays and holidays was collected and sent to the laboratory on the first working day after hospitalization. The cases in which material was collected on the first 5 hospitalization days were analyzed. Laboratory tests were conducted using PCR/RT-PCR for RSV, hMPV, PIV 1, 2, and 3, Flu A and B, and ADV. Oligonucleotide primers were used for each virus (Table 1). RT-PCR assays were performed using the High Capacity cDNA Archive kit (Applied Biosystems, Carlsbad, USA). Amplifications were run separately (not multiplex). After RT-PCR, amplification products (plate column) were purified and transferred to sequencing tubes (Applied Biosystems, Carlsbad, USA). Amplified fragments were analyzed using an ABI Prism 310 genetic analyzer (Applied Biosystems, Carlsbad, USA) and the GeneScan 3.1.2 software.[8]

The study was approved by the Ethics in Research Committee of the University Hospital of Universidade de São Paulo.

Statistical analysis

Categorical variables were analyzed using a chi-square test, and continuous variables, the Mann-Whitney test. The association between explanatory variables and outcomes was analyzed using first univariate and then multivariate logistic regression. For that purpose, continuous outcome variables were treated as binary variables and classified according to their median value. The confounding variables age and breastfeeding duration were entered as continuous variables. These variables were also analyzed as categories. The level of statistical significance was set at $p > 0.05$. The Stata 10.0® software was used for statistical analyses.

Results

Of the 395 infants hospitalized due to acute respiratory infection during the study, 304 were selected, and 291 had

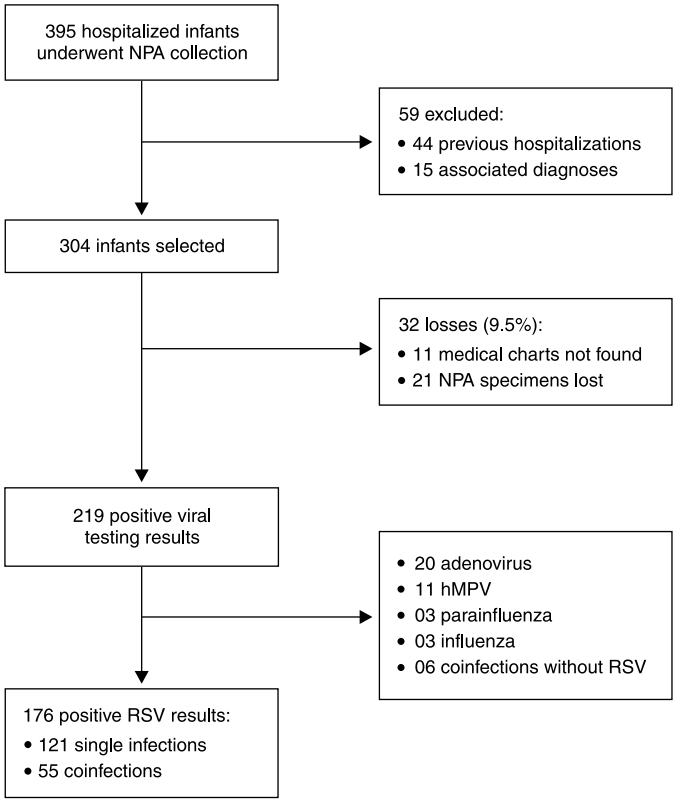
viral infections. RSV was the most frequent virus identified in this group (80.4%). The 176 infections by RSV were analyzed in this study (Figure 1).

Eighty-five per cent of the infants had a diagnosis of bronchiolitis or pneumonia. Other less frequent diagnoses were asthma and persistent or recurrent wheezing (14.8%). Chronic diseases were not frequent: in the group of single RSV infection, there were 2 patients with heart disease, 2 with neuromuscular diseases, and 1 with chronic pulmonary disease; in the group with RSV and coinfections, there was 1 patient with heart disease, and 2 with neuromuscular diseases. The characteristics of the 176 infants are summarized in Table 2.

RSV infections occurred as single infections in 121 infants (68.8%) and as coinfections in 55 infants (31.2%). The most frequent viruses associated with RSV were ADV (43.6%) and hMPV (29.1%). Other coinfections with RSV and different respiratory viruses were less frequent and could not be analyzed statistically because of the small number of cases (Flu A = 4; PIV 3 = 3; PIV 2 = 1; PIV 3 + hMPV = 1; Flu A + ADV = 1; and ADV + hMPV = 5).

Infection severity was analyzed by comparing severity outcomes between groups: single RSV infection (RSV); coinfection by RSV and any other virus (RSVCo); coinfection by RSV and adenovirus (RSV + ADV); and coinfection by RSV and human metapneumovirus (RSV + hMPV). Of the confounding variables, prematurity, age and breastfeeding were determinants of severity (Table 3). Prematurity increased absolute risk of coinfection by any virus, which was 28 and 48% for non-premature and premature patients (absolute risk increase 20%; p = 0.04), and of coinfection by RSV + hMPV, from 9.4% to 26.3% (absolute risk increase = 16.9%; p = 0.04). The increase in absolute risk of coinfection by RSV + ADV was not statistically significant (absolute risk = 16 and 26%; absolute risk increase = 10%; p = 0.26).

Severity outcomes were similar in the RSV, RSVCo, RSV + ADV and RSV + hMPV groups according to univariate analysis. After controlling for confounding factors (prematurity, age and breastfeeding), all severity outcomes remained similar between the RSV, RSVCo, RSV + ADV and RSV + hMPV groups (Table 3).



hMPV = human metapneumovirus; NPA = nasopharyngeal aspirate; RSV = respiratory syncytial virus.

Figure 1 - Flowchart of patient inclusion/exclusion in the study

Table 1 - Polymerase chain reaction assay panel for each virus

Virus/Primer	Gene	Sequences (5′ > 3′)	Size of amplified fragment (bp)
RSV			
VSRAB-F1	F	AACAGTTTAACATTACCAAGTGA	380
VSRAB-R1	TCATTGACTTGAGATATTGATGC		
PIV 1			
HPIV1-F1	HN	CCGGTAATTTCTCATACCTATG	317
HPIV1-R1	CCTTGGAGCGGAGTTGTTAAG		
PIV 2			
HPIV2-F1	HN	CCATTTACCYAAGTGATGGAAT	203
HPIV2-R1	GCCCTGTTGTATTTGGAAGAGA		
PIV 3			
HPIV3-F1	HN	ACTCCCAAAGTTGATGAAAAGAT	102
HPIV3-R1	TAAATCTTGTTGTTGAGATTGA		
Influenza A			
FLUA-F1	NS1	CTAAGGGCTTTCACCGAAGA	192
FLUA-R1	CCCATTCTCATTACTGCTTC		
Influenza B			
FLUB-F1	NS1	ATGGCCATCGGATCCTCAAC	241
FLUB-R1	TGTCAGCTATTATGGAGCTG		
Adenovirus			
ADENO-F1	Hexon	CCC(AC)TT(CT)AACCACCACCG	167
ADENO-R1	ACATCCTT(GCT)C(GT)GAAGTTCCA		
hMPV			
MPV-F1	F	GAGCAAATTGAAAATCCCAGACA	347
MPV-R1	GAAAACTGCCGCACAACATTTAG		

bp = base pairs; hMPV = human metapneumovirus; PIV = parainfluenza virus; RSV = respiratory syncytial virus.

Table 2 - Demographic and medical characteristics of 176 infants included in the study according to absence or presence of coinfections

Characteristics	Total RSV n = 176	Single RSV infectio n = 121	RSV + coinfection n = 55	p
Male sex (%)	100 (56.8)	69 (57.0)	31 (56.3)	0.935*
Age (months)†	4.5 (1.5-8.7)	4.2 (1.45-8.55)	4.6 (1.5-9.5)	0.560‡
Birth weight (g)†¶	3,034 (2,740-3,360)	3,090 (2,778-3,367)	2,980 (2,580-3,300)	0.138‡
Prematurity** (%)	27 (16.8)	14 (11.6)	13 (23.6)	0.119*
Age at weaning†‖	2 (0-4)	2 (0.25-4)	1.5 (0-4)	0.369‡
Hospitalization time (days)†	8 (6-10)	8 (6-10)	8 (6-12)	0.225‡
Oxygen therapy duration (days)†	6 (4-8)	6 (4-8)	6 (4-10)	0.269‡
Intensive care unit admission (%)	57 (32.4)	36 (29.8)	21 (38.2%)	0.268*
Mechanical ventilation (%)	27 (15.3)	17 (14.1)	10 (18.2)	0.481*
Bronchiolitis§ (%)	103 (58.5)	72 (59.5)	31 (56.4)	0.664*
Pneumonia§ (%)	47 (26.7)	30 (24.8)	17 (30.9)	0.664*

RSV = respiratory syncytial virus.
* Chi-square.
† Median (p25 - p75).
‡ Mann-Whitney test.
§ Diagnosis at admission.
‖ Data available for 155 cases.
¶ Data available for 143 cases.
**Data available for 161 cases.

Table 3 - Univariate and multivariate analyses of possible determinants of infection severity

Analysis	Hospitalization time *			O^2 duration*			ICU admission			Mechanical ventilation		
	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
Univariate analysis												
Male sex	0.78	0.42-1.42	0.41	0.90	0.49-1.65	0.74	0.94	0.49-1.77	0.84	1.51	0.66-3.44	0.33
Prematurity	0.45	0.23-0.88	0.02	0.43	0.22-0.83	0.01	0.44	0.22-0.88	0.02	0.32	0.14-0.75	0.01
Age†	0.94	0.89-0.99	0.04	0.91	0.86-0.98	0.01	0.98	0.92-1.04	0.49	0.89	0.81-0.99	0.04
Breastfeeding duration†	0.80	0.65-0.98	0.04	0.78	0.63-0.96	0.02	0.79	0.64-0.98	0.04	0.86	0.65-1.14	0.31
RSVCo	1.32	0.70-2.51	0.39	1.46	0.77-2.78	0.25	1.46	0.75-2.85	0.27	1.36	0.58-3.20	0.48
RSV + ADV	1.62	0.67-3.91	0.28	1.93	0.80-4.66	0.15	1.42	0.57-3.53	0.46	0.87	0.23-3.25	0.84
RSV + hMPV	0.82	0.28-2.41	0.72	1.27	0.44-3.64	0.66	1.07	0.35-3.31	0.90	0.87	0.18-4.19	0.87
Multivariate analysis‡												
Model 1												
RSV	1.00			1.00			1.00			1.00		
RSVCo	0.58	0.21-1.55	0.27	0.88	0.33-2.33	0.80	0.97	0.36-2.60	0.96	1.05	0.29-3.85	0.94
Model 2												
RSV	1.00			1.00			1.00			1.00		
RSV + ADV	0.97	0.23-4.00	0.96	1.21	0.29-5.13	0.79	1.50	0.35-6.42	0.59	1.29	0.21-7.86	0.78
Model 3												
RSV	1.00			1.00			1.00			1.00		
RSV + hMPV	0.21	0.04-1.17	0.07	0.48	0.10-2.21	0.34	0.30	0.05-1.80	0.19	0.88	0.58-1.32	0.54

95%CI = 95% confidence interval; ICU = Intensive care unit; O^2 = oxygen therapy; OR = odds ratio; RSV = respiratory syncytial virus; (n = 121); RSV + ADV = RSV and coinfection by adenovirus (n = 24); RSVCo = RSV and coinfection by any other virus (n = 55); RSV + hMPV = RSV and coinfection by human metapneumovirus (n = 16).
* Dichotomized according to median value.
† Treated as continuous variables.
‡ Outcomes adjusted for prematurity, age and breastfeeding duration.

Discussion

This cohort of hospitalized infants with RSV infection had a high rate of coinfections by other respiratory viruses (31%). Coinfections were not associated with disease severety, regardless of the virus in association with RSV and the presence of any counfounding factor, such as prematurity, age and breastfeeding duration.

Evidence in current literature indicates that the clinical meaning of the simultaneous identification of more than one virus in respiratory secretions is controversial. Cilla et al. also did not find any differences in the prognosis of children infected by one or more viruses according to hospitalization time, ICU admission and oxygen therapy. Other reports found different and conflicting results. Some suggest that severity is greater in viral coinfections, whereas others found greater severity in infections by a single pathogen. Semple et al. found that RSV + hMPV coinfection increased 10 times the relative risk of ICU admission for mechanical ventilation (relative risk = 10.99, 95%CI = 5.0-24.12, $p < 0.001$). As the studies were retrospective, characteristics not evaluated in the populations under study or even differences in viral subtypes and the interaction with environmental factors may explain the differences found.[5,9]

Interesting evidence, though weaker, has been produced by case reports.[10] Greensill et al. evaluated children with bronchiolitis caused by RSV that received mechanical ventilation; 70% had coinfection by hMPV, which suggested greater severity in these cases. In contrast, Canducci found lower severity in cases of coinfection by RSV + hMPV than in infections by a single virus in hospitalized children.[3,10]

Conflicting results may be explained by several factors, such as the fact that different pathogenic mechanisms may be triggered by different viruses that mutually potentialize or mitigate each other's effects. Moreover, the actual pathogenic role of each virus may be unclear. The simultaneous detection of one or more pathogenic virus, such as those investigated in this study, is usually classified as coinfection. However, the presence of viral genome detected using molecular biology techniques may indicate viral persistence, with no significant pathogenic effect at the time of detection.[2,9,10] A recent study evaluated the presence of ADV DNA in respiratory secretions of children that had recurrent infections and found both recurrent infections due to different ADV genotypes and persistence of viral DNA for a long time, which stresses the importance of the correlation between laboratory findings and clinical

signs and symptoms.[11] RSV + ADV coinfection was the most frequent in this study, as well as in studies conducted by other authors, who found coinfection rates of up to 43% when this pathogen was involved.[2] Infections by ADV alone have been frequent in the service where this study was conducted. In a previous study, the authors found that ADV was the second most frequent agent of single infections in hospitalized infants with acute respiratory disease, with prevalence rates ranging from 5.6 to 9.6%. The occurrence of less aggressive genotypes may explain the mild features of ADV infection, but viral genotyping was not performed in this study.[12,13] The overlapping of RSV and hMPV infection seasons, previously demonstrated in a 4-year surveillance study in the same service where this study was conducted, also explains the high RSV + hMPV coinfection rates. Viral coinfections are frequently caused by the pathogens that are predominant in single infections in the period under study.[14] Interestingly, prematurity increased the risk to coinfection by hMPV (absolute risk increase = 16.9%; p = 0.04), but severity remains similar to that of single infection by RSV.

The high prevalence of viral coinfection by ADV and hMPV may also be explained by the characteristics of the study population, composed of infants, most of them in their first year of life, with bronchiolitis and pneumonia, and hospitalized, in particular, during the season of the respiratory viruses.[9] The use of molecular biology methods also had high diagnostic sensitivity, and positive results were found in 72% of the samples. This finding is in agreement with those in the literature, as studies found viral detection rates ranging from 45 to 70% under the same conditions and viral coinfection rates from 15 to 39%.[2-4,15-17]

This study included only hospitalized children, and its results cannot be extrapolated to children with less severe conditions. Tests to detect bocavirus and rhinovirus were not performed. The inclusion of these viruses in this study might have resulted in a greater prevalence of coinfections.[9,18] However, these two pathogens are usually less frequent causes of bronchiolitis and pneumonia in infants than the viruses selected for this study. The role of the rhinovirus is more remarkable, as it is an important trigger of wheezing episodes in atopic children, but is also usually isolated in about 30% of asymptomatic individuals. Moreover, in the same way as the bocavirus, it often has an unclear pathogenic role.[9,18] Had these pathogens been included, the analysis of coinfection prognosis in the study population might not have been different.

This study had some limitations due to its retrospective design. The parameters used were selected because they are carefully evaluated and recorded in the service's charts, and, therefore, data are adequate to assess clinical severity by means of medical chart reviews, not subject to any retrospective interpretation bias, as would be the case with clinical scores. Other factors, such as passive smoking, daycare center attendance, and contact with school age children could not be assessed because these data are not as carefully collected and recorded in medical charts as the variables included in this study. The agreement between the different severity outcomes and the similar clinical progression of patients with infection by RSV as a single agent or coinfection by other virus further support to our results.

Conclusion

Respiratory viral coinfections were frequent in infants hospitalized due to RSV infection. Our results suggest that coinfection of RSV and another virus is not clinically relevant and does not change the prognosis of infection during its acute phase.

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