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Viral Codetection and Clinical Outcomes of Infants Hospitalized With Bronchiolitis: A Multicenter Cohort Study

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Background: The simultaneous identification of multiple respiratory viruses is common in infants hospitalized with respiratory tract infections. Respiratory syncytial virus (RSV) is one of the main pathogens in bronchiolitis, although codetection of rhinovirus, influenza and other respiratory viruses may occur in about one-third of cases. The relevance of viral codetection on disease severity is still controversial. This multicenter cohort study aimed to assess the clinical outcomes of infants under 24 months hospitalized with bronchiolitis, comparing those testing positive for RSV alone, RSV plus another virus and ≥2 viruses distinct from RSV.

Methods: Data were collected across 13 hospitals in Lombardy, Italy, both in the prepandemic and pandemic years. Random effect regression models were also employed to test the association between 3 groups (infants testing positive for RSV alone, RSV plus another respiratory virus and no RSV but ≥2 respiratory viruses other than RSV) and course of bronchiolitis, adjusted for potential confounders.

Results: Among 1788 infants, 86.7% tested positive for RSV alone, 6.9% for RSV plus another virus and 6.3% for ≥2 other viruses. Significant differences were found in clinical outcomes: infants with multiple non-RSV viruses had shorter oxygen supplementation, intensive care and hospital stay compared with those with RSV alone. Notably, codetection of RSV and another virus was associated with a higher risk of radiologically confirmed pneumonia, whereas detection of ≥2 non-RSV viruses was inversely associated with pneumonia.

Conclusions: These findings point out that codetection of viruses other than RSV is associated with milder disease courses than detection of RSV alone in infants with bronchiolitis. On the other hand, patients with RSV and another virus are at higher risk of pneumonia than infants affected by RSV alone. Further research is required to understand the underlying mechanisms and optimize management strategies in infants with bronchiolitis testing positive for multiple viruses.

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BACKGROUND

Several studies have highlighted the prevalence and significance of viral codetections in pediatric patients with respiratory tract infections.[1,2] These codetections, often involving respiratory viruses such as respiratory syncytial virus (RSV), rhinovirus and influenza, occur in a substantial portion of pediatric patients, with reports showing rates ranging from 5% to over 30% in some cohorts.[1–3] However, their potential impact on the clinical outcomes is controversial.[3–5]

Bronchiolitis is a major cause of hospitalization in infants and young children, with RSV being the most frequent pathogen identified.[6] While RSV alone is known to cause significant morbidity, the role of coinfections in disease severity, hospital stay duration and the need for intensive interventions remains controversial.[7–10] The presence of multiple viral pathogens might exacerbate the inflammatory response in infants with bronchiolitis, leading to more severe respiratory symptoms and a prolonged course of illness.[11,12] On the other hand, some reports suggested that viral codetections might be associated with milder presentations and outcomes.[4,13] Because data are conflicting and mainly come from monocenter studies before the COVID-19 pandemic,[13–15] there is a need to further understand the relevance of viral codetection in infants with bronchiolitis.[14]

The primary objective of this multicenter study is to investigate whether infants hospitalized with bronchiolitis who test positive for RSV alone, RSV plus another viral pathogen or ≥2 viral agents distinct from RSV exhibit different clinical trajectories. The secondary aim is to evaluate if these possible different trajectories were similar before and during the COVID-19 pandemic.

MATERIALS AND METHODS

This investigation is part of the IRIDE (Investigating bRonchiolitis epidemiology During the pandemic Emergency) study.[16] Briefly, this multicenter observational cohort study was conducted across 27 hospitals in Lombardy, Northern Italy. It includes infants up to 24 months of age, hospitalized for bronchiolitis during 4 specific periods: prepandemic (July 2018 to March 2019) and 3 pandemic-related periods (July 2020 to March 2021, July 2021 to March 2022 and July 2022 to March 2023). In this study, we selected hospitals that routinely conducted viral testing on nasopharyngeal specimens collected at admission from infants diagnosed with bronchiolitis, targeting the following viruses: RSV, rhinovirus, human metapneumovirus, influenza virus, parainfluenza virus, adenovirus, bocavirus and coronaviruses. Samples were analyzed by polymerase chain reaction in the main laboratory of each hospital. The following further data were considered for the analyses: age, sex, neonatal

gestational age, history of breastfeeding and presence of chronic diseases potentially affecting the bronchiolitis course. Radiological diagnosis of pneumonia, duration of oxygen supplementation, use of noninvasive ventilation, intensive care admission and duration, use of invasive ventilation support and the duration of the hospitalization were considered as outcomes. All data were retrospectively collected and deidentified at each study center. The data were then compiled using REDCap tools hosted at the Ospedale Maggiore, Policlinico of Milan, Italy. The study received approval from the Ethical Committee of the Coordinating Center (Ethical Committee Milano Area 2, Milan, approval code 186796, on April 26, 2023).

Statistical Analysis

Normally distributed continuous variables were reported as mean and SDs and nonnormally distributed ones as median and interquartile range. The distribution of these variables was visually gauged by density plots and histograms. Frequencies and percentages were used to report categorical data. Characteristics and outcomes of the following 3 groups were compared: (1) RSV alone; (2) RSV plus another respiratory virus or (3) no RSV but ≥2 respiratory viruses other than RSV. One-way ANOVA and the Kruskal-Wallis test were employed to compare continuous data. The Fisher exact test or the χ^2 test was employed to compare categorical data, as appropriate. The Bonferroni correction was applied for multiple comparisons.

Then, random effect regression models were performed. The hospital was considered a random effect. The viral infection group (testing positive for only RSV as the reference group) and study period (July 2020 to March 2021 as reference period) and interaction between these 2 variables were used as predictors. All models were adjusted for age, sex, gestational age at birth, history of breastfeeding (yes vs. no) and chronic disease (yes vs. no), which are well-recognized potential confounders of bronchiolitis severity. Models' results are expressed as linear coefficients for continuous outcomes and odds ratio (OR) for categorical outcomes, along with 95% confidence interval and *P* values. $P < 0.05$ was assumed as significant. All analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Among the 2474 potentially eligible infants from 13 hospitals, 686 were excluded because only 1 virus other than RSV was detected, and 1788 were ultimately included in this study. The flowchart of the patient's enrollment is depicted in Figure 1. Males were 981 (54%), and the median age of the cohort was 2.0 (1.0–5.0) months. The mean gestational age at birth was 38.3 (SD, 2.5) weeks. Eighty-four (4.7%) had a chronic disease, and 1094 (76%) had a history of breastfeeding.

A total of 1551 tested positive exclusively for RSV (87%), whereas 124 (6.9%) tested positive exclusively for RSV plus another virus and 113 (6.3%) for ≥2 other respiratory viruses. The characteristics of the patients in the 3 groups are given in Table 1.

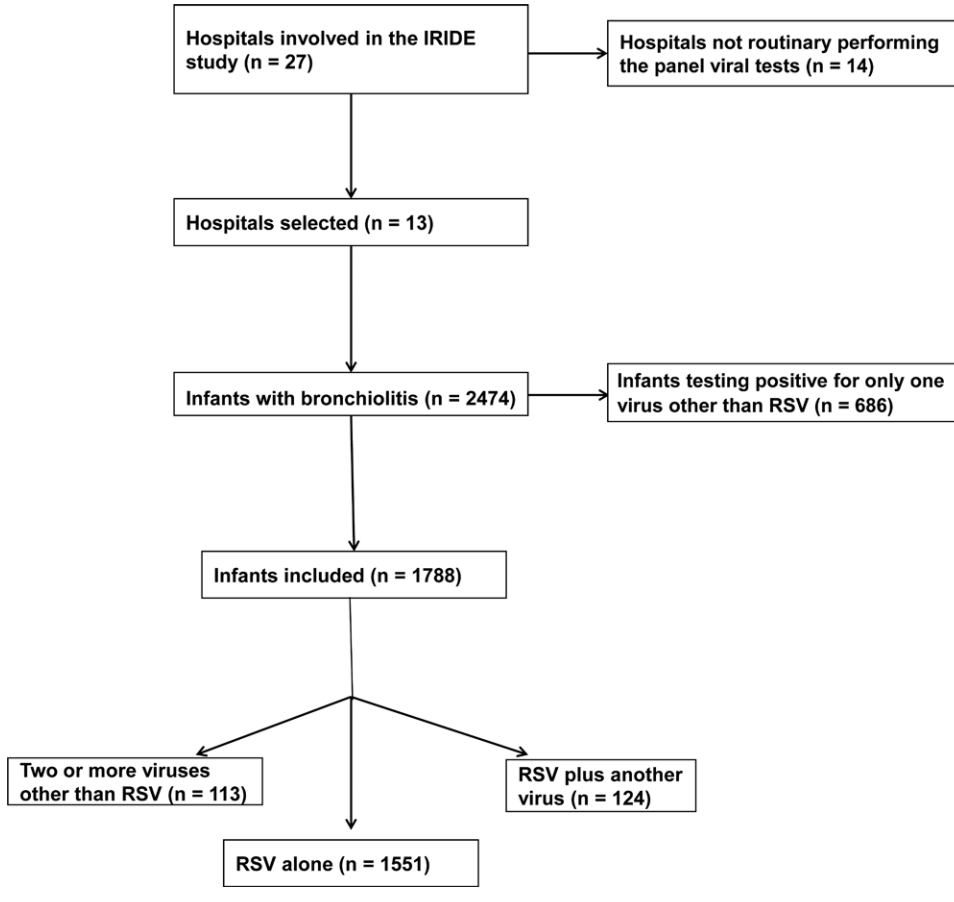

FIGURE 1. Flowchart of patient's inclusion.

TABLE 1. Demographics, Clinical and Microbiological Characteristics of Included Infants in the 3 Study Groups (Testing Positive for RSV Alone, RSV Plus Another Respiratory Virus or ≥2 Respiratory Viruses Other Than RSV). Data Are Presented as Median (Interquartile Range), Mean (±SD) or Absolute Frequency (Percentage)

	All	RSV alone	RSV plus	≥2 viruses (no RSV)	*P*
N	1788	1551	124	113	
Age, mo	2.0 (1.0–5.0)	2.0 (1.0–4.0)	3.5 (2.0–9.3)	5.0 (2.0–11.0)	<0.001
Sex					
Female	807 (45)	695 (45)	62 (50)	50 (44)	0.525
Male	981 (55)	856 (55)	62 (50)	63 (56)	
Neonatal gestational age, wk	38.3 (±2.5)	38.4 (±2.4)	38.2 (±2.8)	37.5 (±3.7)	0.005
Neonatal body weight, grams	3114 (±634)	3126 (±608)	3077 (±733)	2987 (±848)	0.116
History of breastfeeding	1094 (76)	955 (76)	79 (81)	60 (70)	0.231
Length of breastfeeding, mo	2.0 (1.0–4.0)	2.0 (1.0–3.0)	3.0 (1.4–5.5)	2.0 (1.5–4.0)	0.011
Length of exclusive breastfeeding, mo	2.0 (1.0–4.0)	2.0 (1.0–3.0)	2.4 (1.0–4.0)	2.0 (1.1–4.0)	0.039
Chronic disease*	84 (4.7)	65 (4.2)	8 (6.5)	11 (9.7)	0.017

*Chronic disease included: bronchopulmonary dysplasia, severe congenital cardiopathy, cystic fibrosis, Down syndrome, congenital diaphragmatic hernia, neuromuscular diseases, immunodeficiency, storage disorders, esophageal atresia or pulmonary transplantation.

TABLE 2. Bronchiolitis Course and Outcome in the 3 Study Groups (Testing Positive for RSV Alone, RSV Plus Another Respiratory Virus or ≥2 Respiratory Viruses Other Than RSV). Data Are Presented as Median (Interquartile Range) or Absolute Frequency (Percentage)

	RSV alone	RSV plus	≥2 viruses (no RSV)	*P*
N	1551	124	113	
Radiological diagnosis of pneumonia	351 (23)	48 (39)	22 (20)	<0.001
Length of oxygen supplementation, d	3 (1–6)	4.0 (3–7)	3 (1–5)	<0.001
Noninvasive ventilation support	831 (54)	86 (69)	57 (50)	0.002
Intensive care unit admission	437 (28)	39 (32)	23 (20)	0.133
Invasive ventilation support	46 (3.0)	2 (1.6)	1 (0.9)	0.309
Length of intensive care stay, d	5 (4–8)	4 (3–7)	4 (2–6)	0.048
Length of the whole hospitalization, d	7 (5–9)	8 (6–10)	6.5 (4–9)	0.001

Unadjusted analyses (Table 2) showed a difference among the 3 groups for the occurrence of radiologically confirmed pneumonia ($P < 0.001$), duration of oxygen supplementation ($P < 0.001$), use of noninvasive ventilation ($P = 0.002$) and duration of the intensive care admission ($P = 0.048$) and hospitalization ($P = 0.001$). No difference was found between intensive care admission and the use of invasive ventilation support.

The results of multiple regression models provided partially similar results. In particular, testing positive for ≥2 viruses other than RSV was associated with a shorter duration of oxygen supplementation by 1.4 days ($P < 0.001$), with a lower duration of intensive care by 0.8 days ($P = 0.035$) and with a lower duration of hospitalization by 1.1 days ($P = 0.039$). No differences were observed for the other outcomes. Radiologically confirmed pneumonia was inversely associated with the presence of ≥2 respiratory viruses other than RSV (OR, 0.40; $P = 0.007$) and positively associated with RSV plus another pathogen (OR, 2.13; $P = 0.001$). Testing positive for RSV plus another virus, compared with testing positive for RSV alone, was not associated with any other difference in the outcomes. The details of mixed effects model results are reported in Tables 3 and 4 for continuous and categorical outcomes, respectively. The interaction between the study period and infection groups did not provide any significant association in any model; therefore, we did not consider the interaction term in the final models.

DISCUSSION

This large cohort study aimed to elucidate the impact of viral codetection on the clinical outcomes of infants hospitalized with bronchiolitis, with a particular focus on distinguishing between infections caused by RSV alone, RSV plus another virus and multiple viruses other than RSV. Furthermore, it tested if different clinical trajectories characterized the period before the SARS-CoV-2 pandemic and 3 pandemic periods.

This study showed a high prevalence of RSV in infants hospitalized for bronchiolitis, as documented in previous studies. In line with earlier research, we also observed notable differences in clinical characteristics and outcomes among infants testing positive for different viruses.[14,17,18] Infants testing positive for multiple viruses other than RSV showed a lower duration of oxygen supplementation, intensive care stay and overall hospitalization compared with infants with RSV alone. These findings partially challenge the traditional assumption that viral coinfections usually worsen disease severity.[17,19,20] This outcome could be attributed to complex immune responses triggered by the interaction of multiple viruses, potentially leading to a reduced inflammatory response to the infection.[13] On the other hand, previous studies have reported conflicting results regarding the effect of infection by RSV alone or RSV plus another virus.[9,21–23] This variability could be attributed to differences in study design, patient populations and the specific viruses involved in the disease.[3] This study, which included a large number of hospitalized infants, supports the view that the presence of 1 additional virus alongside RSV may not usually result in increased disease severity.

A particularly unexpected result from this study was the inverse association between the presence of multiple non-RSV viruses and the occurrence of radiologically confirmed pneumonia. An opposite association was found comparing infants with RSV plus another pathogen and those with RSV alone. This finding raises questions about the mechanisms by which different viral combinations interact with the host's immune system and respiratory

TABLE 3. Results of the Mixed Effect Regression Models: Length of Intensive Care Unit Stay, Oxygen Supplementation and Overall Hospitalization Were the Dependent Variables. Study Groups (Testing Positive for RSV Alone, RSV Plus Another Respiratory Virus or ≥2 Respiratory Viruses Other Than RSV) and Study Periods (Reference 2018–2019). Models Were Adjusted for Age, Sex, Gestational Age at Birth, Chronic Disease (Yes vs. No), History of Breastfeeding, Number of Older Siblings (No Older Sibling vs. ≥1 Older Sibling) and Testing Positive for Respiratory Syncytial Virus

Outcome	Predictive variable	β	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Length of intensive care unit stay					
	RSV plus	0.082	-0.560	0.724	0.802
	≥2 viruses (no RSV)	−0.764	−1.474	−0.054	0.035
	2020–2021	0.467	−1.259	2.193	0.596
	2021–2022	−0.453	−0.862	−0.043	0.030
	2022–2023	0.028	−0.397	0.454	0.896
Length of oxygen supplementation					
	RSV plus	0.245	−0.427	0.916	0.475
	≥2 viruses (no RSV)	−1.384	−2.127	−0.641	0.000
	2020–2021	0.177	−1.628	1.983	0.847
	2021–2022	0.321	−0.107	0.750	0.142
	2022–2023	0.597	0.152	1.042	0.009
Whole duration of hospital stay					
	RSV plus	0.647	−0.277	1.572	0.170
	≥2 viruses (no RSV)	−1.106	−2.155	−0.058	0.039
	2020–2021	2.598	0.106	5.090	0.041
	2021–2022	−0.295	−0.906	0.316	0.344
	2022–2023	0.562	−0.072	1.195	0.082

Table 4. Results of the Mixed Effect Regression Models: Radiological Diagnosis of Pneumonia, Noninvasive Ventilation Support, Invasive Ventilation Support and Intensive Care Admission Were the Dependent Variables. Study Groups (Testing Positive for RSV Alone, RSV Plus Another Respiratory Virus or ≥2 Respiratory Viruses Other Than RSV) and Study Periods (Reference 2018–2019). Models Were Adjusted for Age, Sex, Gestational Age at Birth, Chronic Disease (Yes vs. No), History of Breastfeeding, Number of Older Siblings (No Older Sibling vs. ≥1 Older Sibling) and Testing Positive for Respiratory Syncytial Virus

Outcome	Predictive variable	Odds ratio	Lower 95% confidence interval	Upper 95% confidence interval	*P*
Radiological diagnosis of pneumonia					
	RSV plus	2.133	1.350	3.372	0.001
	≥2 viruses (no RSV)	0.400	0.205	0.780	0.007
	2020–2021	1.247	0.313	4.975	0.754
	2021–2022	0.786	0.566	1.090	0.148
	2022–2023	0.766	0.548	1.069	0.117
Noninvasive ventilation					
	RSV plus	1.257	0.776	2.037	0.352
	≥2 viruses (no RSV)	0.703	0.403	1.228	0.216
	2020–2021	2.205	0.571	8.507	0.251
	2021–2022	1.785	1.294	2.464	<0.001
	2022–2023	3.149	2.232	4.443	<0.001
Invasive ventilation					
	RSV plus	0.455	0.098	2.119	0.316
	≥2 viruses (no RSV)	0.168	0.020	1.412	0.101
	2020–2021	4.338	0.375	50.195	0.240
	2021–2022	0.382	0.161	0.903	0.028
	2022–2023	0.841	0.389	1.819	0.660
Need for intensive care unit admission					
	RSV plus	1.528	0.919	2.540	0.102
	≥2 viruses (no RSV)	0.668	0.333	1.344	0.258
	2020–2021	2.942	0.704	12.305	0.139
	2021–2022	0.774	0.552	1.086	0.139
	2022–2023	1.035	0.724	1.480	0.849

epithelium. It is possible that certain viral combinations may limit the replication of pathogens in the lower respiratory tract, modify microbiome or directly modulate immune responses against secondary bacterial infections, such as pneumonia.[24,25] Overall, these data suggest that infants testing positive for RSV plus another pathogen should be particularly monitored for the possible higher risk of pneumonia.

In the context of the COVID-19 pandemic, our study also provides insights into the potential impact of pandemic-related changes in viral epidemiology and host immune responses on bronchiolitis outcomes. Several studies have reported shifts in the epidemiology of viral infections in infants during the pandemic, including the phenomenon of "immune debt."[26,27] Our findings suggest that while pandemic-related changes may have influenced

the overall incidence and severity of bronchiolitis cases, the clinical trajectories of infants with different viral infection profiles remained consistent across study periods.

The strengths of this study include its multicenter design, large sample size and rigorous statistical analyses, which allowed for robust comparisons between the different infection groups. However, several limitations must be acknowledged. The observational nature of the study precludes definitive conclusions about causality. Although we adjusted for several known confounders of bronchiolitis severity, residual confounding may still be present. The study was limited to inpatients, and it was not possible to ascertain changes in viral tests during the hospitalization because infants were tested only once. Although the main viruses responsible for bronchiolitis were investigated, we cannot exclude the presence of some other viruses. Similarly, the potential occurrence of bacterial superinfection was not addressed. The use of chest x-rays is not a routine exam according to national guidelines.[28] It is possible that some infants with pneumonia did not undergo this examination. Therefore, the generalizability of our results on pneumonia cannot be extended to infants with a diagnosis of pneumonia based only on clinical presentation. Given the multicenter nature of the study, different laboratories performed the virus tests. On the other hand, analyses were performed in laboratory hospitals, which undergo systematic independent controls to adhere to international standards for analyses. Finally, this study investigated viral codetection, which is not synonymous with viral coinfection.

In conclusion, this large cohort study pointed out that infants with viral codetection without RSV may experience shorter hospital stays and less severe disease courses than those with RSV. Further research to explore the underlying mechanisms of viral interactions and their clinical implications is needed.

APPENDIX

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