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Is viral coinfection a risk factor for severe lower respiratory tract infection? A retrospective observational study

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Abstract

Objective: To determine whether viral coinfection is a risk for severe lower respiratory tract infection (LRTI).

Working Hypothesis: Children with viral coinfection had a higher risk for admission to the intensive care unit (ICU) than those with a single virus infection.

Study Design: Retrospective, observational study for 10 years.

Patient-Subject Selection: Children between 1 and 60 months of age hospitalized with LRTI.

KEYWORDS

influenza virus, intensive care unit, lower respiratory tract infection, respiratory syncytial virus, rhinovirus

1 | INTRODUCTION

Viral lower respiratory tract infection (LRTI) presents with an acute, self-limiting, and uncomplicated infection in healthy children, with a significant cause of public health problems and health care costs in those with any known risk factor.[1] In the United States (US), pneumonia and bronchiolitis, generally associated with viral agents, are the most common causes of LRTI-associated hospitalizations in children under 5 years.[2] Moreover, between 2002 and 2009, the estimated hospital charges for children with bronchiolitis younger than 2 years of age increased from 1.3 to 1.7 billion.[3]

With the advances in molecular technology, multiple viruses have been determined at a rate of 30%–70% in children hospitalized with LRTI.[4,5] However, the clinical implications of viral coinfection remain unclear and controversial reports have been published. While Richard et al.[6] found that infants with the dual respiratory virus were 2.7 times higher at risk of admission to the intensive care unit (ICU) than those with single-virus infection, Brand et al.[7] showed no increased risk of severe bronchiolitis in infants with multiple viruses. Despite the small sample size, our previously published study suggested that the presence of influenza A and human bocavirus (HBoV) coinfection were independently a risk for invasive mechanical ventilation (IMV) support.[8] Some meta-analyses showed a comparable effect on hospital admission, the length of hospital stay, and oxygen requirements or death between children with a single virus and viral coinfection.[5,9] In contrast, a systemic review determined that further research has needed to clarify the impact of viral coinfection on the severity of LRTI because of conflicting results in studies.[10]

Considering these controversial reports, we conducted an observational study to investigate the effect of virus coinfection on LRTI in hospitalized children under 5 years of age. These findings may identify the degree to which clinicians should consider viral coinfection when assessing children's risk of developing severe illness.

2 | METHODS

2.1 | Study design, sites, and subjects

We performed a single-center retrospective study of children under 5 years of age hospitalized with a clinical diagnosis of LRTI,

presenting to the emergency department, outpatient clinics (pediatric pulmonology, pediatrics, pediatric infectious disease), or ICU of Ege University Medical Faculty Children Hospital from January 2010 through January 2020. The hospital institutional review board approved this study.

The study institution's standard practice is to screen all admitted patients with respiratory symptoms for viral respiratory pathogens. Because the International Classification of Diseases discharge codes might be inaccurate coding or misdiagnosis on the part of the treating clinician, cases were identified by evaluating the virology laboratory database. Subsequently, we cross-checked the virology laboratory results with electronic medical records and nurse observation charts. According to the revised World Health Organization guidelines,[11] patients between 1 and 60 months of age who clinically fulfilled the criteria of LRTI (pneumonia or bronchiolitis) were included in the study. Exclusion criteria included hospitalization within the preceding 30 days, other diagnoses than LRTI caused by respiratory viruses, hospital-acquired viral LRTI, primary (defined as bacterial growth detected in cultures from sterile cavities such as blood, cerebrospinal fluid, urine, and pleural fluid) or secondary (defined as bacterial growth in cultures taken from sterile cavities 48 h after hospitalization)[12] bacterial infections, newborns (under 1 month of age), and incomplete medical records. Bacterial cultures were obtained from only children with a clinical suspicion of a bacterial infection.

2.2 | Data collection for risk factors, clinical course, and outcomes

We reviewed risk factors, clinical course, and outcomes of all patients eligible for the study. The potential risk factors for severe LRTI regarding the literature[7,13–17] were abstracted from the electronic medical record. The age, gender, prematurity (<37 weeks), history of atopy (clinician-confirmed atopy), exposure to tobacco smoke (smokers in the household), malnutrition status (assessed with weight-for-height *SD* score [SDS])[18] the number and type of virus, underlying condition (immunosuppression [IS], neuromuscular disease [NMD], chronic lung disease [CLD], hemodynamically significant congenital heart disease [CHD], and previous history of LRTI), neutrophil and lymphocyte status for age,[19] C-reactive protein (CRP) value, oxygen requirement (defined as peripheral oxyhemoglobin saturation <92%), and admission to the ICU (defined as those admitted if they required high-flow nasal cannula oxygen [HFNCO]/bilevel positive airway pressure [BiPAP]/IMV) or short term unit were collected. Children with multiple underlying conditions were included in the group considered having a higher risk for underlying disease. Patients with IS/NMD considered the highest-risk, followed by CLD, CHD, and then the previous history of LRTI.

2.3 | Viral diagnostic testing

Respiratory specimens were obtained from children within the first 48 h after the admission and transported to the laboratory in a viral transport medium (UTM, Copan Diagnostics). The automated nucleic acid extraction was performed according to the manufacturer's recommendations using the EZ1 Virus Mini Kit v2 (Qiagen). The RT-PCR conducted on the Rotor-Gene device (Qiagen) with the FTD® Respiratory Pathogens 21 Kit was used to detect respiratory viruses (RSV-A/B, influenza-A/B, HRV, human coronavirus [hCoV] NL63/229E/OC43/HKU1, PIV1-4, HMPV-A/B, HBoV, human adenovirus [HAdV], human enterovirus [EV], and human parechovirus). The kits and results obtained were tested using the National External Quality Assurance Schemes (NEQUAS) External Quality Control program (NEQUAS, Scotland).

2.4 | Statistical analysis

The data were analyzed using the statistical program package SPSS (Version 22.0; IBM Corp.) and were presented as median (interquartile ranges) or number (%). The categorical variables were compared between groups using the χ^2 or Fisher Exact test. The differences in the median of the continuous variables were tested with the two-sided Mann–Whitney or Kruskal–Wallis test.

The primary outcome was to determine the relationship between viral coinfection and severe LRTI. We assessed the severity of the disease according to the site of the hospital stay: the ICU (severe LRTI) or the short-term unit (mild LRTI). The odds ratios (ORs) for viral coinfection, the primary explanatory variable, were calculated by binary logistic regression with a priori adjustment for age (categoric variables).

First, virus infection type was categorized as; (i) single virus infection (reference level); (ii) viral coinfection; (iii) no-virus infection. Subsequently, subgroup analyses by each of the viruses were performed to detect whether a specific virus coinfection led to severe LRTI than this specific-virus infection alone. The 13 models of comparing single and coinfection for each of the viruses were categorized as; (i) specific-virus single infection (reference level); (ii) specific virus and any virus coinfection; (iii) single infections not including this specific virus; (iv) coinfections not including this specific virus; (v) no-virus infection. Finally, we conducted subgroup analyses by age for viral coinfections. Variables with $p < .05$ in bivariate analyses were selected for all multivariate regression models. The weight-for-height SDS, history of atopy, exposure to tobacco smoke, underlying conditions (categorized as healthy [reference level], CLD, CHD, NMD, IS, and previous history of LRTI), neutrophil (categorized as; normal range [reference level], neutropenia, and neutrophilia) and lymphocyte (categorized as; normal range [reference level], lymphopenia, and lymphocytosis) status, and CRP value were included in all model as the confounders. Although the age was entered separately into the model as a continuous and categorical variable (categorized as; 1–3 months, 4–6 months, 7–12 months, 13–24 months, and 25–60 months [reference level]), the ORs of variables were expressed by using the categorized age. Also, dummy variables to represent each level of qualitative independent variables were included in the regression equation. The Hosmer-Lemeshow test was used to assess

the goodness of fit for the model. Results were expressed as ORs with 95% confidence intervals (CIs), and $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Study population

We evaluated 7278 respiratory specimens during the study period. Afterward, we reviewed the patients' medical records and included 2115 (29.2%) children in the study. Of these, mild and severe LRTI were observed in 1553 (73.4%) and 562 (26.6%) children, respectively. A total of 795 (10.9%) patients with a diagnosis other than LRTI, 2200 (30.2%) not fulfilled criteria of LRTI according to the WHO guideline, 834 (11.4%) with hospital-acquired viral LRTI, 212 (2.9%) with a primary bacterial infection, and 1074 (14.8%) with a secondary bacterial infection were excluded from the study (Figure 1). Overall, the most common detected viruses were RSV (906; 42.8%) and HRV (733; 34.7%), followed by HBoV (212; 10.0%). We identified that HRV (226; 40.2% vs. 507; 32.6%; $p = .001$), influenza A (73; 13.0% vs. 136; 8.8%; $p = .004$), and PIV3 (47; 8.4% vs. 84; 5.4%; $p = .01$; Table S1) were observed more frequently in severe disease compared with mild disease.

3.2 | Viral coinfection

Viral coinfection was present in 28.3% (599) of all children, and those with viral coinfection were at a higher risk of severe disease than those with a single virus infection (43.8% vs. 22.7%; adjusted odds ratio [aOR]: 3.44; 95% CI: 2.74–4.53; $p < .001$; Table 1). In subgroup analyses for each virus comparing single virus infection and its coinfection; RSV (aOR: 3.70; 95% CI: 2.62–5.23; $p < .001$), HRV (aOR: 3.50; 95% CI: 2.45–5.02; $p < .001$), influenza A (aOR: 4.83; 95% CI: 2.37–9.84; $p < .001$), PIV3 (aOR: 9.03; 95% CI: 3.73–21.90; $p < .001$), HBoV (aOR: 2.37; 95% CI: 1.27–4.63; $p = .01$), HAdV (aOR: 3.41; 95% CI: 1.27–9.12; $p = .01$), and EV (aOR: 5.06; 95% CI: 1.35–18.89; $p = .01$; Figure 2) coinfections were associated with an increased risk for severe disease.

3.3 | Subgroup analyses by age

Respiratory syncytial virus and HRV (except for between 25 and 60 months) coinfections with any virus were independently associated with severe disease in all age groups (Figures 3–5; Figures S1 and S2). While children between 4 and 24 months had a higher risk of severe infection related to PIV3 coinfection

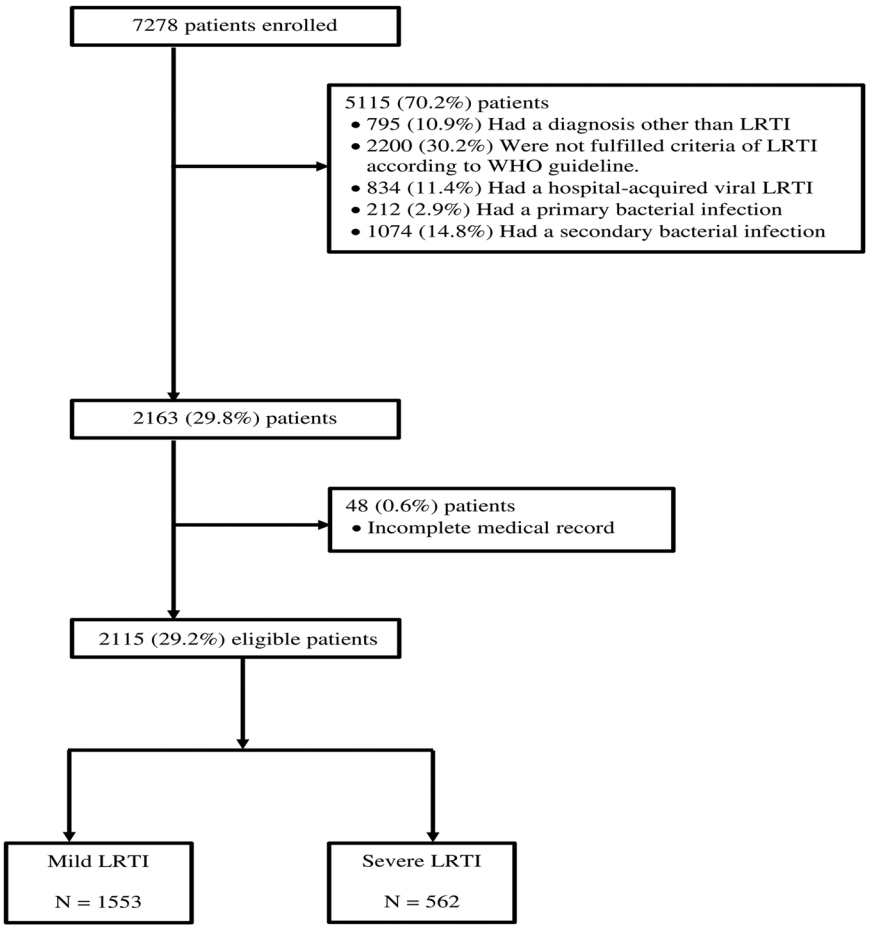

FIGURE 1 The study flow diagram

TABLE 1 Baseline characteristics and risk factors of severe LRTI in children under 5 years of age

Variables	Total patients (*n* = 2115)	Mild disease (*n* = 1553)	Severe disease (*n* = 562)	Unadjusted OR	*p* Value	Adjusted OR	*p* Value
Age (months)	8.0 (4.0-20.0)	8.0 (3.0-20.0)	9.0 (4.0-20.0)	1.01 (0.99–1.02)	.08	0.98 (0.97–0.99)	.01
Age (months)					.02		
1–3	528 (25.0)	411 (26.5)	117 (20.8)	0.78 (0.58–1.06)		1.94 (1.32–2.87)	.001
4–6	347 (16.4)	250 (16.1)	97 (17.3)	1.07 (0.77–1.47)		1.73 (1.16–2.58)	.006
7–12	426 (20.1)	316 (20.3)	110 (19.6)	0.96 (0.70–1.30)		1.44 (1.01–2.06)	.048
13–24	404 (19.1)	275 (17.7)	129 (23.0)	1.29 (0.95–1.75)		1.66 (1.18–2.35)	.004
25–60	410 (19.4)	301 (19.4)	109 (19.4)	Reference		Reference	Reference
Gender				NI	.95	NI	NI
Male	1289 (60.9)	947 (61.0)	342 (60.9)				
Female	826 (39.1)	606 (39.0)	220 (39.1)				
Weight-for-height SDS	0.13 (−0.95–1.03)	0.22 (−0.81–1.15)	−0.18 (−1.37–0.61)	0.82 (0.77–0.87)	<.001	0.89 (0.84–0.96)	.001
Prematurity					<.001		
No	1650 (78.0)	1241 (79.9)	409 (72.8)	Reference		Reference	Reference
Yes	465 (22.0)	312 (20.1)	153 (27.2)	1.48 (1.19–1.86)		1.32 (1.02–1.72)	.03
History of atopy					<.001		
No	1780 (84.9)	1356 (87.3)	424 (75.5)	Reference		Reference	Reference
Yes	301 (14.5)	188 (12.1)	113 (20.1)	1.92 (1.48–2.48)		1.69 (1.27–2.26)	<.001
Missing	34 (1.6)	9 (0.6)	25 (4.4)	–		–	–
Exposure to tobacco smoke					.02		
No	1212 (57.3)	913 (58.8)	299 (53.2)	Reference		Reference	Reference
Yes	903 (42.7)	640 (41.2)	263 (46.8)	1.25 (1.03–1.52)		1.32 (1.06–1.65)	.01
Underlying disease	817 (38.6)	510 (32.8)	307 (54.6)	2.46 (2.02–2.99)	<.001		
Healthy	1298 (61.4)	1043 (67.2)	255 (45.4)	Reference		Reference	Reference
Previous history of LRTI	213 (10.1)	152 (9.8)	61 (10.9)	1.64 (1.18–2.27)		1.58 (1.07–2.34)	.02
CLD	199 (9.4)	127 (8.2)	72 (12.8)	2.31 (1.68–3.19)		2.15 (1.46–3.15)	<.001
IS	175 (8.3)	105 (6.8)	70 (12.5)	2.72 (1.95–3.80)		2.63 (1.78–3.87)	<.001
CHD	118 (5.6)	67 (4.3)	51 (9.1)	3.11 (2.11–4.59)		3.51 (2.29–5.39)	<.001
NMD	112 (5.3)	59 (3.8)	53 (9.4)	3.67 (2.47–5.45)		3.91 (2.51–6.09)	<.001
Type of infection					<.001		
Single	1473 (69.6)	1173 (75.5)	300 (53.4)	Reference		Reference	Reference
Coinfection	599 (28.3)	353 (22.7)	246 (43.8)	2.72 (2.21–3.34)		3.44 (2.74–4.53)	<.001
None-virus	43 (2.0)	27 (1.7)	16 (2.8)	0.81 (0.23–4.35)		0.76 (0.23–4.92)	.4
Neutrophil status					<.001		
Normal range	1227 (58.0)	939 (60.4)	288 (51.2)	Reference		Reference	Reference
Neutrophilia	702 (33.2)	469 (30.2)	233 (41.5)	1.63 (1.33–2.00)		1.71 (1.34–2.19)	<.001
Neutropenia	177 (8.4)	136 (8.8)	41 (7.3)	0.99 (0.68–1.44)		0.93 (0.61–1.41)	.75
Missing	9 (0.4)	9 (0.6)	0 (0)	–		–	-

TABLE 1 (Continued)

Variables	Total patients (n = 2115)	Mild disease (n = 1553)	Severe disease (n = 562)	Unadjusted OR	p Value	Adjusted OR	p Value
Lymphocyte status					<.001		
Normal range	1070 (50.6)	773 (49.7)	297 (52.8)	Reference		Reference	Reference
Lymphocytosis	826 (39.1)	647 (41.7)	179 (31.9)	0.72 (0.58–0.90)		0.68 (0.53–0.87)	.002
Lymphopenia	210 (9.9)	124 (8.0)	86 (15.3)	1.82 (1.34–2.47)		1.88 (1.33–2.65)	<.001
Missing	9 (0.4)	9 (0.6)	0 (0)	–		–	–
CRP value	0.7 (0.2–2.2)	0.07 (0.2–2.08)	1.0 (0.3–3.0)	1.06 (1.03–1.09)	<.001	1.05 (1.01–1.08)	.002

Note: Data are number (%), median (interquartile range), or odds ratio (95% confidence interval). Age was included in the model regardless of the p value. Gender was not included in the model because of its p value more than .05. N = 2115 patients, within 38 missing values; 2077 patients were included in the multivariate analysis (adjusted R^2 = .25). Neutrophil and lymphocyte status was categorized as their reference range for age.

Abbreviations: CRP, C-reactive protein; LRTI, lower respiratory tract infection; NI, not include; OR, odds ratio; SDS, *SD* score.

(Figures 3–5 and Figure S1), those between 7–12 and 25–60 months had severe illness associated with influenza A coinfection (Figure 5 and Figure S2). HBoV coinfection was independently a risk factor for severe disease in children between 7 and 12 months (Figure 5). However, we did not analyze the subgroup analysis for EV coinfection because of its small sample size.

3.4 | Potential risk factors

We showed that old age (aOR: 0.98; 95% CI: 0.97–0.99) reduced severe disease risk after the confounders adjusted. Children with higher weight-for-height SDS were significantly associated with mild disease (aOR: 0.89; 95% CI: 0.84–0.96). Moreover, prematurity, history of atopy, exposure to tobacco smoke, underlying condition, neutrophilia, lymphopenia, and high CRP value were independent risks for severe disease (Table 1).

4 | DISCUSSION

This study reported that children between 1 and 60 months with viral coinfection increased the risk of admission to the ICU for HFNCO/BiPAP/IMV assistance. While RSV and HRV (except for between 25 and 60 months) coinfections were associated with severe disease in all ages, PIV3 (7–24 months) and HBoV (7–12 months) coinfections led to severe LRTI in early childhood. Besides, our study showed that influenza A coinfection was independently a risk factor for severe LRTI in children between 7–12 and 25–60 months.

In our study, children with viral coinfection constituted 28.3% of the study population, and HBoV was the most common virus after RSV and HRV. Previous studies conducted in Barcelona and Rome,[20,21] where the Mediterranean climate is dominant, like Izmir, had similar results to that presented here. On the contrary, other studies reported from Canada and Japan,[7,22] where the continental and subtropical climate is dominant, found that viral coinfection was 17.2% and 15.8% of the patients, with the third common virus as influenza A and HMPV, respectively. These results emphasize that the seasonal distribution and frequency of viruses may vary in different countries according to their geographical and meteorological characteristics. Furthermore, the annual circulation of infections and the composition of the study population, and the method used in virus detection, may also affect the viral coinfection rate. For example, the studies conducted in infants or hospitalized patients may determine a higher viral coinfection rate than those held in adults or population-based studies because of the more frequent detection of multiple-viruses in these patients.[21] Therefore, the authors should interpret the viral coinfection rate carefully among various studies. Children with viral coinfection were 3.4 times more at risk of admission to the ICU than those with a single virus infection, concordant with the previous studies.[6,23] Several possible explanations for these results are that the direct interactions among viral genes or gene products and host immune system, the indirect interactions of host environmental changes, and the immunological interactions may determine the course of LRTI in children with viral coinfection.[24,25] The first virus may boost viral superinfection by consuming host defense, similar to bacterial infection.[26] Moreover, the coinfection of two distinct virus types may have affected the natural course of LRTI in children.

Although HRV generally causes a common cold in children,[27] it reduces cell proliferation and bronchial epithelial cells' self-repair capacity.[28] Thus, any secondary virus infection may increase the risk of severe illness. Besides, HRVs are a large group of genetically diverse RNA viruses, and HRV subtypes, particularly HRV-A and -C, have been shown to cause severe LRTI in children.[29] Unfortunately, we could not determine HRV subtypes in the respiratory specimens because of our virus detection

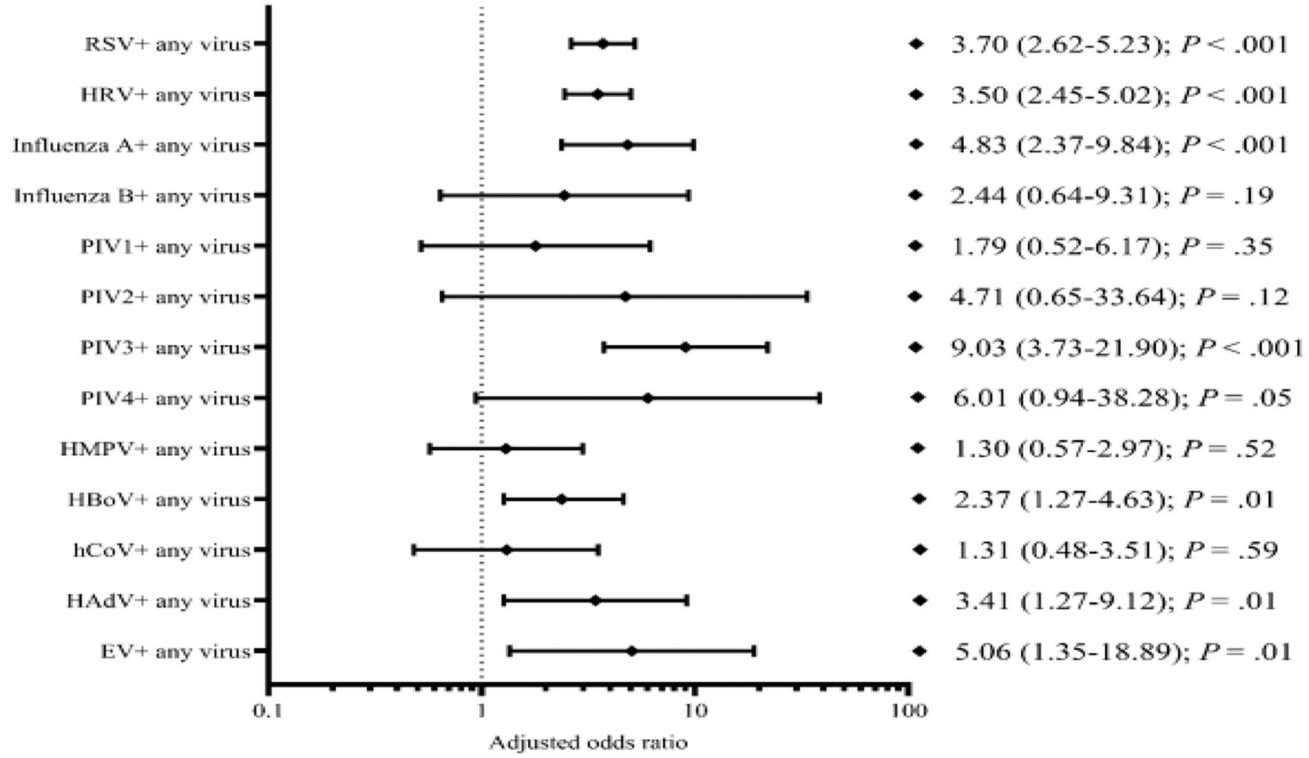

FIGURE 2 Comparison of virus coinfection with single virus infection

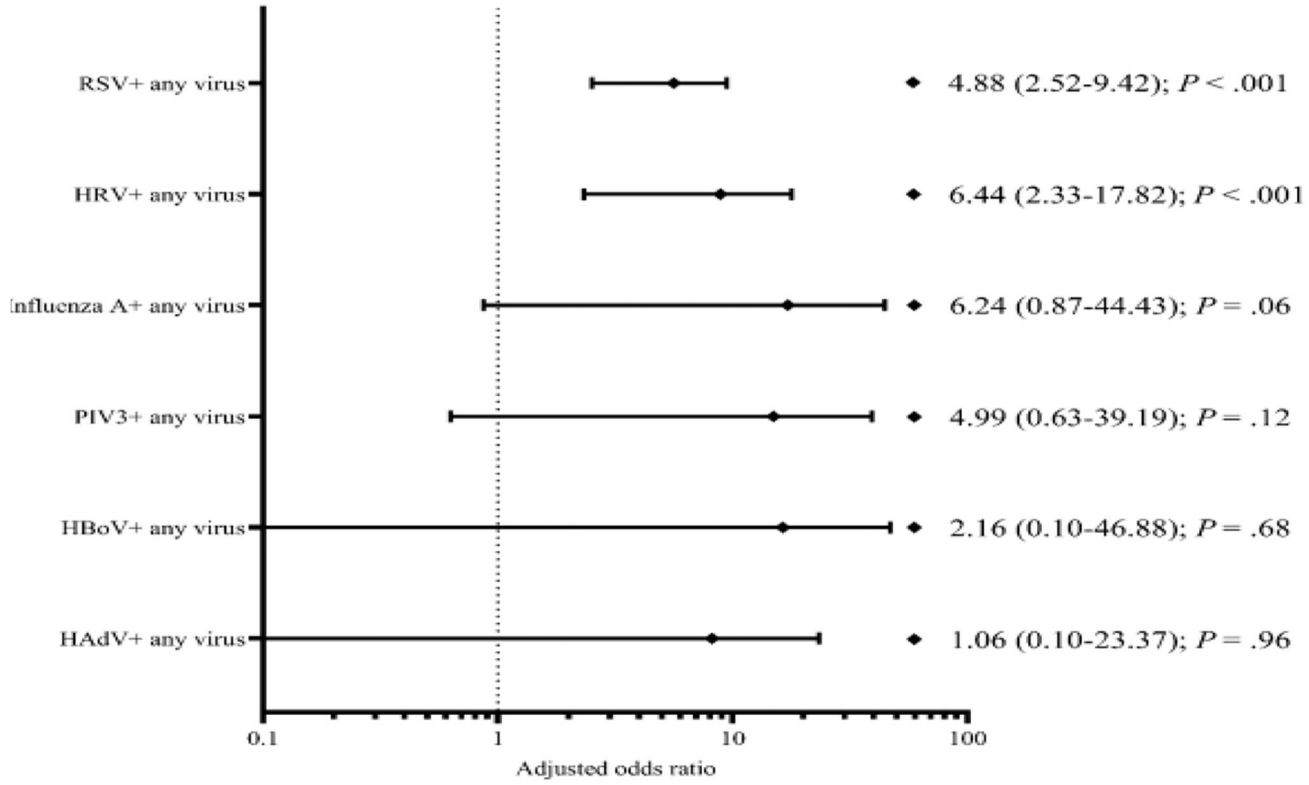

FIGURE 3 Subgroup regression analysis of each virus coinfection by age between 1 and 3 months

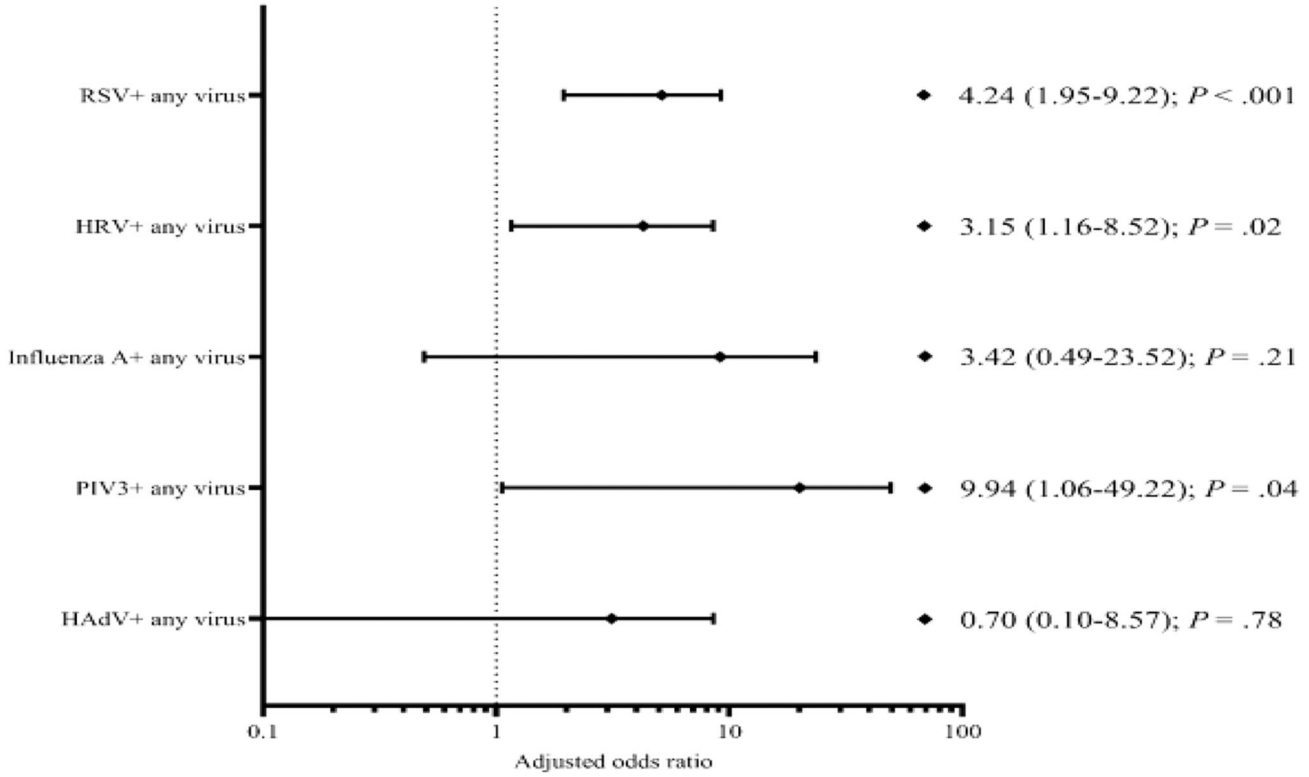

FIGURE 4 Subgroup regression analysis of each virus coinfection by age between 4 and 6 months

method. These subtypes might have infected some severe cases. This issue stands as an interesting subject for further research. Richard et al.[6] found that infants with RSV coinfection were at 2.7 times higher risk of admission to the ICU, while Semple et al.[23] determined a 10-fold higher risk of IMV in children under 2 years of age who had RSV and HMPV coinfection. Additionally,

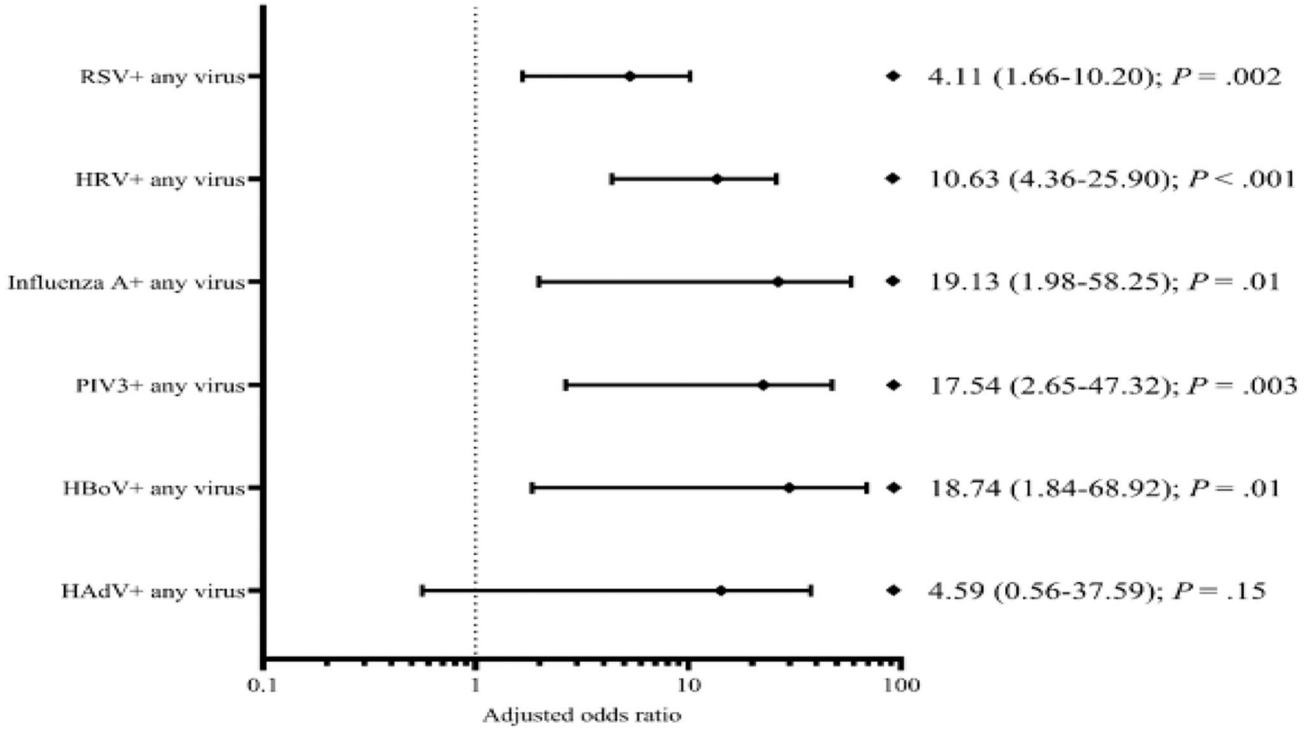

FIGURE 5 Subgroup regression analysis of each virus coinfection by age between 7 and 12 months

our results suggested that RSV coinfection remains an essential agent for severe LRTI not only in children younger than 24 months but also in those beyond 24 months.

Although coinfection with other viruses is very common during influenza infection,[30] secondary bacterial infections may exist at a rate of 40% in LRTI associated with influenza A.[31,32] Therefore, we note that any secondary bacterial infection, which was not detected, might increase influenza A's severity. However, a comprehensive and extensive sample size study should be needed. Although there is no licensed influenza vaccine for children under 6 months, alternative strategies, including maternal vaccination during pregnancy and household vaccination, might reduce severe influenza infections. Increasing influenza and bacterial vaccination, with environmental precautions such as frequent hand washing, decontamination of hands, and cleaning concrete surfaces with water and disinfectants, are essential to prevent transmission of respiratory viruses and reduce the severe disease burden. The efficacy of neuraminidase inhibitors in healthy children is limited and does not recommend general treatment.[33] Nevertheless, early initiation of neuraminidase inhibitors is associated with shorter symptoms, decreased complications, and hospitalization.[33]

HBoV was first identified in children's respiratory tract in 2005; 75% of the HBoV infections are associated with multiple viral infections.[16] HBoV infection usually results in a mild, self-limiting respiratory tract infection and might even be asymptomatic.[4] However, several case reports reported that HBoV coinfection with other viruses could cause complications such as pneumothorax, pneumomediastinum, and severe respiratory failure requiring ICU/IMV.[34] Slow elimination of the viral antigens by the immature immune system might explain the coinfection with HBoV and the severity of the illness in infants. Considering the National Respiratory and Enteric Viruses Surveillance System study conducted from 1990 to 2004,[35] PIV3 (52%) was the most frequently determined serotype among PIVs. In the US, the estimated LRTI and hospitalization related to PIV3 were reported at 1.1 million and 29,000, respectively.[36] Parainfluenza virus 3 leads to LRTI more common than other serotypes in neonates and infants and is clinically indistinguishable from RSV infection. Additionally, PIV and RSV belong to the Paramyxoviridae family, enveloped RNA, similar epidemiologic and clinical outcomes.[22] Thus, children aged younger than 24 months with PIV3 coinfection may need equal medical attention to those with RSV coinfection regarding disease severity.

Our study's limitations include those related to retrospective studies, including bias regarding patient selection and accuracy related to the medical record. To minimize bias, we developed a standardized data form to guide data collection, only included patients with the medical records information, and the same experienced clinician performed data collection. Second, patients who visit the hospital later may present with more severe signs and symptoms than those who visit the hospital immediately after illness onset. However, we could not compare this potential effect on disease severity because of the study's retrospective design. Third, including only children presented to the tertiary hospital might have resulted in the study population's heterogeneity. Finally, a reliable test for bacterial codetection was not available at the time of the study, which could cause that we might have underestimated the impact of bacteria on severe LRTI.

In conclusion, children between 1 and 60 months hospitalized with LRTI and detected viral coinfection were at about 3.5 times higher risk for HFNCO/BiPAP/IMV assistance. Respiratory syncytial virus and HRV (except for between 25 and 60 months) coinfections caused severe LRTI in all age groups, whereas PIV3 (4–24 months) and HBoV (7–12 months) coinfections were associated with severe LRTI in early childhood. Additionally, influenza A coinfection led to severe LRTI in children between 7–12 and 25–60 months.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Aykut Eşki: conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (lead); validation (equal); visualization (equal); writing original draft (equal); writing review & editing (equal). **Candan Çiçek**: conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing original draft (equal); writing review & editing (equal). **Figen Gülen**: conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); writing original draft (equal); writing review & editing (equal). **Esen Demir**: conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); writing original draft (equal); writing review & editing (equal).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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