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Original Article

The Impact of Multiple Viral Infection in Children with Severe Lower Respiratory Tract Infections

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ABSTRACT

Aim: We aimed to compare the clinical features and outcomes between single and multiple viral pathogens in children with severe lower respiratory tract infections (LRTIs) in a pediatric intensive care unit (PICU).

Materials and Methods: This study was conducted retrospectively in patients who were admitted to a PICU between March, 2018 and March, 2020. The subjects were divided into two groups, single viral infection and multiple viral infection. The epidemiologic characteristics, clinical features, disease severity and outcomes were compared between these single and multiple viral infection groups.

Results: During this study period, positive polymerase chain reaction (PCR) tests were carried out on 136 (29%) children among the 468 children admitted to the PICU with the diagnosis of LRTI. Rhinovirus and Respiratory Syncytial Virus (RSV) were the most commonly identified viruses (44.1% and 35.2%, respectively). Two viruses were detected in thirty-nine (28.6%) of samples via PCR tests. Rhinovirus and RSV co-infection was the most common combination (10/39, 25.6%) in our cohort. The multiple viral infection group had higher PRISM scores than the single virus infection group (10 vs. 7, respectively, p=0.009). In the multiple viral infection group, the invasive ventilatory support rate (56.4% vs 36.1%, p=0.030) and the non-invasive ventilatory (NIV) support rate (43.5% vs 6.1%, p=0.018) were significantly higher than in the single viral infection group.

Conclusion: Lower respiratory multi-viral infections are associated with increased invasive and NIV support requirements. Close monitoring in a unit where support can be provided is essential for those infants with multi-viral LRTIs.

Keywords: Respiratory viruses, children, co-infection, critical care, lower respiratory tract infections

Introduction

Viral lower respiratory tract infections (LRTIs) are major causes of hospitalization for children under 2 years of age. The most frequent respiratory virus identified during hospitalization in children admitted to the pediatric intensive care unit (PICU) is Respiratory Syncytial Virus (RSV). Rhinovirus/enterovirus, Influenza A/B, Coronavirus, Parainfluenza virus, human Metapneumovirus (hMPV) and Bocaviruses are the other common viral causes of LRTIs. In recent years, improvements in molecular techniques including multiplex polymerase chain reaction (PCR) tests have allowed for the detection of viral pathogens with a wide spectrum. In children with LRTIs, PCR tests detected viral pathogens in up to 95% of cases. PCR test results are positive in up to 40% for viral co-infections (1-3).

In the literature, there are controversies regarding the relationship between viral co-infection and disease severity. In studies including children who were admitted to PICUs, viral co-infection was not associated with invasive

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mechanical ventilation requirements and/or mortality (4,5).

In this study, we aimed to compare the clinical features and outcomes between single and multiple viral pathogens in children with LRTIs in a PICU.

Materials and Methods

This study was a retrospective and comparative study of patients admitted to the PICU at Ege University Children's Hospital, a tertiary intensive care unit with 17 beds. We identified children aged between 1 month and 18 years with severe LTRI who had undergone PCR tests. From March 1st, 2018 to March 1st, 2020, 1,170 patients were admitted to the PICU and 490 (41.8%) had a primary diagnosis of severe LRTI. Four hundred and sixty-eight (40%, 468/1,170) children who had undergone PCR tests were included in this study.

Detailed clinical data on each patient were collected from secure electronic medical records. This included demographic and clinical characteristics; age, sex, gestational age, laboratory results, Pediatric Risk of Mortality Score (PRISM), radiological findings, and outcome data including; length of PICU stay, type of respiratory support, length of respiratory support, positive bacterial lower respiratory tract co-infection and mortality. Bacterial co-infection was defined as the identification of a bacterial pathogen in culture from an endotracheal specimen in those children who had an endotracheal tube. Tracheal aspirate samples were obtained from ventilated patients through an endotracheal tube by direct tracheal aspiration. Those patients who required mechanical ventilation with negative admission endotracheal aspirate sampling but tested positive after the second day of intubation were determined to be ventilatory-associated pneumonia and they were excluded from this study. Pediatric acute respiratory distress syndrome was defined according to the Pediatric Acute Lung Injury Consensus Committee criteria: namely, findings of new infiltrate(s) consistent with acute pulmonary parenchymal disease and PaO_2/FiO_2 (P/F) ratio <300 or oxygen saturation/FiO_2 (S/F) ratio ≤265 (6).

The subjects were divided into two groups. The single viral infection group had only one virus reported via PCR, and the multiple viral infection group had two or more viruses reported via PCR.

This study was approved by the Clinical Research Ethics Committee of Ege University Faculty of Medicine (20-9.1T/48). The patients and/or parents of patients agreed to participate in this study after being informed of its purpose.

Respiratory virus examination by polymerase chain reaction test samples

Samples were taken from all children by a nasopharyngeal swab or an endotracheal aspirate in patients who were intubated within the first 24 hours of PICU admission. The samples were transported to the laboratory for PCR tests in a suitable container.

Viral nucleic acid isolation and amplification

Nucleic acids were isolated from the clinical samples with EZ1 Viruses Mini Kit v2.0 (Qiagen, Germany) protocol in EZ1 Advanced (Qiagen, Germany). The nucleic acids were stored at minus 80 degrees until amplification. Viral nucleic acid amplification was carried out with Fast Track Diagnostics (FTD) Respiratory Pathogen 21 tests FTD, Luxemburg) by means of multiplex real-time PCR. Influenza A, influenza B, influenza A, Rhinovirus, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, Coronavirus HKU1, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, hMPV A/B, Human bocavirus, Mycoplasma pneumoniae, RSV A/B (RSV), Adenovirus, Enterovirus and Human parechovirus can be determined with FTD Respiratory Pathogen 21 test. Amplifications of multiplex real-time PCR were determined with measurement of fluorescence radiation in Rotor-Gene (Qiagen, Germany). If the samples had a fluorescence signal, they were accepted as positive.

Statistical Analysis

The data were analyzed using the SPSS version 17.0 software. Conformity of variables to the normal distribution was examined with analytical methods such as the Kolmogorov-Smirnov/Shapiro-Wilk tests. Categorical data are presented as percentages, numerical data with Gaussian distribution are presented as mean ± standard deviation, and abnormally distributed data are presented as median (interquartile range). The Mann-Whitney U test was used to compare binary groups (single virus group vs. multiple virus group) in continuous data. Pearson's chi-square or Fisher's Exact test was used in the analysis of categorical data. Statistical results were considered significant for p-values <0.05.

Results

From March, 2018 to March, 2020, 490 children were admitted to the PICU with diagnoses of LRTI and 468 of these patients (468/490, 95.5%) who had a PCR test were enrolled into this study. Positive PCR tests were seen in 136 (29%, 136/468) of these patients. Of the 136 specimens included in this study, 91 (66.9%) were nasopharyngeal

swabs and 45 (33.1%) were endotracheal aspirates. As can be seen in Table I, Rhinovirus and RSV were the most commonly identified viruses (44.1% and 35.2%, respectively) in our cohort. Two viruses were detected in thirty-nine (28.6%) of the samples via PCR tests. Among the 39 children with viral co-infections, Rhinovirus was identified in 24 (61.5%) samples. Rhinovirus and RSV co-infection was the most common combination (10/39, 25.6%) in our cohort; followed by Rhinovirus and Enterovirus (6/39, 15.3%); Rhinovirus and Bocavirus (4/39, 10.2%); and RSV and Coronavirus (3/39, 7.6%). Bacterial co-infection was identified by endotracheal culture in 23 patients (16.9%). The most commonly detected organisms included *Streptococcus pneumoniae* (9 patients), *Staphylococcus aureus* (6 patients), *Haemophilus influenzae* (5 patients), *Moraxella catarrhalis* (2 patients), and *Pseudomonas aeruginosa* (1 patient). There was no statistically significant difference in the rate of confirmed bacterial infection between the single and multiple viral infection groups. The rate of empirical use of antibiotics was 76.2%.

The median age of the patients was 9 months [interquartile range (interquartile range IQR), 28.7 months]. One hundred and twenty-one patients (92%) were under 5 years of age and 77 patients (56.6%) were under 1 year of age. Seventy-nine of the 136 patients (58.1%) were male and 57 of the 136 children (41.9%) had an underlying medical condition, most commonly cardiovascular or neurologic diseases. The demographic and clinical characteristics of the patients are presented in Table II.

Fifty-seven children (42%) required intubation prior to PICU admission. Invasive ventilation was required in 57 children (41.9%) with a median duration of mechanical ventilation of 6 days (range, 1-41 days). Non-invasive ventilatory (NIV) support (either high-flow nasal cannula or bilevel positive airway pressure) was required in 23 children (16.9%). The median length of PICU stay was 5 days (range, 1-60 days). One patient (0.7%) required extracorporeal membrane oxygenation support. The median PRISM score was 9 (IQR 10), and 7 (5%) of the patients died during their PICU stay.

Table III shows the comparison between the single and multiple viral infection groups for clinical variables. There was no statistically significant difference in age, sex, chronic disease and mortality between the single and multiple viral infection groups. The multiple viral infection group had higher PRISM scores than the single virus infection group (10 vs. 7, respectively, p=0.009). In the multiple viral infection group, the invasive ventilatory support rate (56.4% vs. 36.1%, p=0.030) and the NIV support rate (43.5% vs. 6.1%, p=0.018) were significantly higher than in the single viral infection group.

Table I. Pathogens in single and multiple viral lower respiratory tract infections in pediatric intensive care unit

Microbiology result	Single infection (n)	Co-infection (n)	Total (n, %)
Rhinovirus	35	25	60 (44.1)
RSV	31	17	48 (35.2)
Bocavirus	5	9	14 (10.2)
Influenza A	8	5	13 (9.5)
Parainfluenza	6	5	11 (8)
Coronavirus	2	8	10 (7.3)
Enterovirus	0	6	6 (4.4)
Adenovirus	1	4	5 (3.6)
Influenza B	4	1	5 (3.6)
hMPV	4	0	4 (2.9)

RSV: Respiratory Syncytial Virus, hMPV: Human Metapneumovirus

Table II. Study population characteristics (n=136)

Characteristics	n (%) or median (interquartile range)
Gender, male	79 (58.1)
Age, months	9 (28.7)
<1 year	77 (56.6)
<5 years	121 (92)
Underlying disease	57 (41.9)
Cardiac	21 (15.4)
Neurologic	12 (8.8)
Respiratory	12 (8.8)
Immunocompromised	7 (5.1)
Prematurity	5 (3.6)
PRISM score	9 (10)
PICU length of stay, (days)	5 (8.7)
Hospital length of stay, (days)	7 (10.7)
Duration of invasive ventilation (days)	6 (7.5)
PARDS, n (%)	12 (8.8)
Bacterial co-infection, n (%)	23 (16.9)
Antibiotic treatment, n (%)	78 (57.3)
Antiviral treatment, n (%)	37 (27.2)
Mortality, n (%)	7 (5.1)

PRISM: Pediatric risk of mortality, PICU: Pediatric intensive care unit, PARDS: Pediatric acute respiratory distress syndrome

Table III. Comparison of clinical and disease severity variables between single and multiple viral infection group

	Single viral infection (n=97)	Multipl viral infection (n=39)	p-value
Gender, male. n (%)	52 (53.6)	27 (69.2)	0.095
Age, months, median (IQR)	9 (27.7)	6 (33)	0.334
Underlying disease, n (%)	42 (43.2)	15 (38.4)	0.605
Non-invasive ventilatory support, n (%)	6 (6.1)	17 (43.5)	**0.018**
Invasive ventilatory support, n (%)	35 (36.1)	22 (56.4)	**0.030**
Invasive ventilation days, median (IQR)	5 (7)	8 (9.25)	0.325
PICU length of stay, median day (IQR)	9 (12)	11 (14.75)	0.410
Hospital length of stay, median day (IQR)	12 (15)	14 (20.5)	0.524
PRISM score, median (IQR)	7 (10.5)	10 (12)	0.009
PARDS, n (%)	8 (8.2)	4 (10.3)	0.742*
Bacterial co-infection, n (%)	17 (17.5)	6 (15.4)	0.763
Mortality, n (%)	5 (5.2)	2 (5.1)	1.000*

*Fisher's exact test
IQR: Interquartile range, PICU: Pediatric intensive care unit, PARDS: Pediatric acute respiratory distress syndrome, PRISM: Pediatric risk of mortality

Discussion

In this study, we showed that multiple viral LRTIs were associated with higher rates of invasive and NIV support requirements and higher PRISM scores at admission. However, there was no statistically significant difference in PICU length of stay and mortality between the single and multiple viral infection groups. Although there are controversial reports about the association between multiple viral infections and disease severity, this may be related to heterogeneities in the patient populations and disease severity definitions (7-10). In PICU specific studies, no association has been reported between viral co-infection and clinical outcomes, including the need for mechanical ventilation and mortality (4,5).

Consistent with the previous studies, our study revealed that the prevalence of multiple viruses was 28.6% (4,7,11,12). Rhinovirus and RSV co-infection was the most common combination (25.6%) in our cohort, as has been reported previously (5,7). It is well known that RSV is one of the main agents associated with upper and LRTI in infants and it has been shown to cause more serious illness than other respiratory viruses (13). The association between specific co-infections and disease severity has been reported in previous studies. Semple et al. (8) reported that the hMPV and RSV co-infection is associated with severe bronchiolitis and it resulted in a 10-fold increase in the risk of PICU admission. Mansbach et al. (14) reported that those children with RSV/Rhinovirus co-infections had significantly longer lengths of hospital stay in comparison to children with RSV-only infections. On the other hand, in a study which compared the disease severity between single and multiple viruses, the authors reported that infants with RSV alone had longer lengths of hospital stay in comparison to those with RSV/Rhinovirus co-infection (15). It has been reported in the literature that milder Rhinovirus infections may have a protective effect (16).

Although there are conflicting results on the association between specific viral co-infections and disease severity, it is important to be aware that children with multiple viral infections may need invasive/non-invasive ventilatory support. Richard et al. (7) reported that infants with viral co-infection were 2.9 times more likely to be admitted to PICUs than those with single viral infections. In a previous study, they demonstrated that, in children with severe bronchiolitis, the use of early NIV resulted in an effective PCO_2 reduction and speculated that early NIV support prevents airway collapse and disease progression (17). In this patient population, early NIV support in the pediatric ward or pediatric emergency department may decrease PICU admissions. In another study which screened children <1 year of age who were admitted to a PICU, no differences were reported in comorbidities between single and multiple virus infections, which is consistent with our findings (18).

Epidemiological studies are generally conducted among symptomatic children by respiratory viral panels. In a large scale community study, the rates of asymptomatic respiratory virus infection were reported to be between 69% and 74% among different age categories (19). The rate of Rhinovirus colonization prevalence in the nasopharynx of asymptomatic children was between 5% and 18% (20,21). While respiratory virus panels allow us to identify treatable pathogens such as influenza, high rates of asymptomatic infection may lead to misjudgment.

In PICUs, approximately 50% of antibiotic use is inappropriate, and antibiotic overuse results in antibiotic resistance, increased costs, and drug toxicities (22).

Previous studies have demonstrated that a high percentage of children received empirical antibiotics (up to 100% in intubated children with bronchiolitis) (23,24). Respiratory virus examination with the PCR method not only provides epidemiological data, but also guides us on the use of oseltamivir. However, it does not affect the decision to use antibiotics. Although there are molecular methods which can be used for viral-bacterial infection differentiation and antimicrobial treatment selection, they have not been able to take their place in clinical practice due to their high cost and low accessibility (25).

Chauhan and Slamon (10) reported that multi-viral infections had a higher association of culture positive bacterial infection in children who required invasive ventilation and a higher rate of radiologic pneumonia. Our results are consistent with studies reporting no differences in confirmed bacterial infection rates in those case of dual infections (18). In our study, the presence of bacterial co-infection was evaluated only in intubated children, it may explain our report of lower bacterial infection rates.

Study Limitations

There were several limitations to this study, mainly attributed to its retrospective design. The presence of bacterial co-infection was evaluated only in intubated children, considering that the clinical definition of bacterial infection may lead to selection bias.

Conclusion

Lower respiratory multi-viral infections are associated with increased invasive and NIV support requirements. Close monitoring in a unit where support can be provided is essential for infants with multi-viral LRTIs. In the future, investigations which aim to identify the association between unfavorable outcomes and specific co-infections are needed.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Ege University Faculty of Medicine (20-9.1T/48).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: P.Y.Ö., B.K., Design: P.Y.Ö., B.K., Data Collection or Processing: H.F.A., İ.E., Analysis or Interpretation: C.Ç., Writing: P.Y.Ö.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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