ORIGINAL RESEARCH ORİJİNAL ARAŞTIRMA

DOI: 10.5336/pediatr.2024-107706

Association of RSV, Influenza in Acute Bronchiolitis Patients Admitted to a Pediatric Emergency Department: Cross-Sectional Descriptive Research

Bir Pediatrik Acil Servise Başvuran Akut Bronşiyolit Hastalarında RSV, İnfluenza Birlikteliği: Kesitsel Tanımlayıcı Araştırma

Özlem ÖZCANLI ÇAY[a], Özlem KEMER AYCAN[a]

[a]Balıkesir University Faculty of Medicine, Department of Pediatrics, Balıkesir, Türkiye

ABSTRACT Objective: We examined the frequency of respiratory syncytial virus (RSV) and influenza co-infection in patients with acute bronchiolitis who visited the pediatric emergency department, and their effects on the course of the disease by evaluating their nutritional status using the Prognostic Nutritional Index (PNI) score. **Material and Methods:** A retrospective cohort study was conducted at Balıkesir Atatürk City Hospital between January 2022-January 2024. A total of 400 patients aged 1-24 months with acute bronchiolitis were included in the study. Patients were classified into 3 groups: RSV positive (Group 1), influenza positive (Group 2), and co-infection (Group 3). Patients were divided into normal (PNI ≥45) and poor (PNI <45) nutritional status groups. **Results:** A total of 400 patients were analyzed: 240 (60%) RSV positive, 100 (25%) influenza positive, and 60 (15%) co-infection cases. In the co-infection group, 46.7% of patients had poor PNI scores (<45). In the co-infection group, the proportion of patients with low PNI score was 46.7% and hospitalization rate was 71.4%, intensive care unit requirement was 21.4% and continuous positive airway pressure requirement was 32.1% (p<0.05). Patients with low PNI scores had significantly longer hospitalization (7.4±2.8 days) compared to those with normal PNI scores (4.6±1.8 days) (p<0.05). **Conclusion:** The PNI score proved to be a crucial indicator for predicting patient outcomes in bronchiolitis cases. The combination of RSV and influenza infections in patients who have inadequate nutrition leads to longer hospital stays and requires more respiratory support and results in higher hospitalization rates.

Keywords: Acute bronchiolitis; respiratory syncytial virus; influenza virus

ÖZET Amaç: Çalışmamızda, pediatri acil servisine başvuran akut bronşiyolit hastalarında respiratuar sinsityal virüs (RSV) ve influenza koenfeksiyon sıklığını ve Prognostik Beslenme İndeksi (PNI) skoru kullanarak beslenme durumlarını değerlendirerek, bunların hastalık seyrine etkilerini inceledik. **Gereç ve Yöntemler:** Balıkesir Atatürk Şehir Hastanesinde, Ocak 2022-Ocak 2024 tarihleri arasında retrospektif bir kohort çalışması gerçekleştirildi. Çalışmaya, 1-24 aylık akut bronşiyolitli toplam 400 hasta dâhil edildi. Hastalar 3 gruba ayrıldı: RSV pozitif (Grup 1), influenza pozitif (Grup 2) ve koenfeksiyon (Grup 3). Hastalar, normal (PNI ≥45) ve zayıf (PNI <45) beslenme durumu gruplarına ayrıldı. **Bulgular:** Toplam 400 hasta analiz edildi: 240 (%60) RSV pozitif, 100 (%25) influenza pozitif ve 60 (%15) koenfeksiyon olgusu. Eş zamanlı enfeksiyon grubunda, hastaların %46,7'sinin PNI skoru düşüktü (<45). Eş zamanlı enfeksiyon grubunda, düşük PNI skoru olan hastaların oranı %46,7, hastaneye yatış oranı %71,4, yoğun bakım ünitesi gereksinimi %21,4, sürekli pozitif hava basıncı gereksinimi ise %32,1 idi (p<0,05). Düşük PNI skoru olan hastalar (7,4±2,8 gün), normal PNI skoru olanlara (4,6±1,8 gün) kıyasla anlamlı olarak daha uzun süre hastanede yatmışlardır (p<0,05). **Sonuç:** PNI skorunun, bronşiyolit olgularında hasta sonuçlarını tahmin etmek için önemli bir gösterge olduğu kanıtlanmıştır. Yetersiz beslenen hastalarda RSV ve influenza enfeksiyonlarının birleşimi, daha uzun hastanede kalış sürelerine yol açar, daha fazla solunum desteği gerektirir ve daha yüksek hastaneye yatış oranlarına neden olur.

Anahtar Kelimeler: Akut bronşiyolit; respiratuar sinsityal virüs; influenza virüsü

Correspondence: Özlem ÖZCANLI ÇAY
Balıkesir University Faculty of Medicine, Department of Pediatrics, Balıkesir, Türkiye
E-mail: ozlemozcanli@yahoo.com

Peer review under responsibility of Turkiye Klinikleri Journal of Pediatrics.

Received: 20 Dec 2024 ***Received in revised form:*** 21 Sep 2025 ***Accepted:*** 01 Oct 2025 ***Available online:*** 14 Oct 2025

Acute bronchiolitis is a serious lower respiratory tract infection characterized by inflammation of the small airways that is common in children under 2 years of age.[1] The disease is mainly caused by viral agents, with a marked increase especially in winter and spring.[2] The diagnosis of bronchiolitis is usually based on clinical findings. The most common symptoms include wheezing, tachypnea, chest tightness, nasal breathing and cough. On physical examination, diffuse rhonchi and fine rales may be heard.[3] It is very important for clinicians to predict the prognosis and determine the treatment plan in this disease, which can be severe especially in infancy.[4]

Respiratory syncytial virus (RSV) is the most common causative agent of bronchiolitis, responsible for approximately 60-80% of all cases.[5] Research conducted globally shows RSV causes the most acute lower respiratory tract infections which resulted in 3.6 million hospitalizations during 2019.[6] The influenza virus occurs less frequently in bronchiolitis than RSV but continues to be a significant cause of the disease during winter months since it accounts for about 10% of pediatric bronchiolitis cases as either the main or contributing pathogen.[7] The condition of bronchiolitis in infants and young children is most commonly caused by the RSV but other viral agents such as human metapneumovirus and rhinovirus and adenovirus can also cause it although less frequently.[8,9] Research shows that children with viral bronchiolitis frequently present with multiple viral agents at once which leads to more severe illness and extended hospitalization periods.[10] The prognosis of patients depends on their nutritional condition and immune response together with their viral infection status. The Prognostic Nutritional Index (PNI) serves as a vital tool to assess nutritional and immune health in patients.

The study investigated how often RSV and influenza viruses co-infected patients with acute bronchiolitis who needed emergency department care at the pediatric facility and examined how co-infection affected their medical results. The study evaluated PNI score effectiveness for predicting disease severity and treatment progression in patients. The research aims to establish how viral co-infections together with nutritional status affect bronchiolitis treatment outcomes to develop better patient care approaches in our clinical environment.

MATERIAL AND METHODS

STUDY POPULATION AND SAMPLE

In this research, a retrospective cohort study design was implemented for data collection in the Pediatric Emergency Department of Balıkesir Atatürk City Hospital from January 2022 to January 2024. All patients between 1-24 months of age with a diagnosis of acute bronchiolitis during the study period were screened, and 400 consecutive patients meeting inclusion criteria were enrolled. The patients included in the study were a total of 400 who were between the ages of 1 month and 24 months, and had an admitting diagnosis of “acute bronchiolitis”. As this was a retrospective study including all eligible patients during the study period, formal sample size calculation was not performed; however, post hoc power analysis showed 80% power to detect a 20% difference between groups with our sample size. Inclusion criteria were being between 1-24 months of age, having a diagnosis of acute bronchiolitis, having no history of chronic disease and not being on continuous medication such as bronchodilators, corticosteroids, or antibiotics. Exclusion criteria included age younger than 1 month or older than 24 months, immunosupression, prematurity, congenital heart disease and neurologic diseases. Patients with co-infection with other respiratory viruses (adenovirus, rhinovirus, human metapneumovirus) detected by multiplex polymerase chain reaction were also excluded to focus specifically on RSV and influenza. Patients with more than 20% missing data were excluded from the study, and multiple imputation method was used in patients with less than 20% missing data. Sample selection was based on consecutive patient enrollment.

Study Procedures

The medical records of the patients were retrospectively analyzed. Demographic data (age, gender, place of residence), socioeconomic status (parental education level, monthly household income) were ob-

tained from hospital admission records and patient files. Laboratory findings were recorded in detail.

Laboratory parameters included the following:

- Complete blood count: Hemoglobin, hematocrit, white blood cells (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), platelets.
- Biochemistry: Aspartate transaminase, alanine aminotransferase, blood urea nitrogen, creatinine, sodium, potassium.
- Inflammatory markers: C-reactive protein (CRP) levels.

Nasopharyngeal swab samples were taken for the detection of viral agents and RSV and influenza antigens were evaluated by rapid test methods. Disease severity was categorized into three groups as mild, moderate and severe based on clinical findings. According to the bronchiolitis severity classification described in the most recent clinical pathway, mild disease (score 0-3) included patients with mild tachypnea and wheezing without significant respiratory distress; moderate disease (score 4-8) included patients with moderate tachypnea, diffuse wheezing, and intercostal retractions; and severe disease (score 9-12) included patients with severe respiratory distress, marked retractions, and oxygen saturation <90%.[11] Clinical findings included tachypnea, wheezing, nasal breathing and intercostal retraction. In addition, the need for hospitalization, the need for intensive care and the use of respiratory support [continuous positive airway pressure (CPAP)/mechanical ventilation] were detailed.

The PNI scoring system served to assess nutritional condition and immune system function for all patients. PNI score was calculated using the formula: PNI=10×serum albumin (g/dL)+0.005×total lymphocyte count (/mm^3).[11] The threshold value for PNI was determined as 45 based on previous pediatric studies.[11] Patients were divided into 2 subgroups as good prognosis group with PNI ≥45 and poor prognosis group with PNI <45. RSV positive, influenza positive and co-infection groups were each evaluated according to PNI score. Vaccination status including routine childhood vaccines and seasonal influenza vaccination was recorded from immunization cards when available; however, complete vaccination data was available for only 65% of patients.

Intervention/Treatment Protocol

The intervention group was not specifically defined and all patients were treated according to standard clinical practice protocols. Treatment strategies were individualized according to disease severity and clinical status of the patients. In cases of mild bronchiolitis, symptomatic treatment was applied, nasal aspiration and humidified oxygen supplementation were used to prevent hypoxemia and improve respiratory comfort. In moderate and severe bronchiolitis cases, bronchodilator therapy (nebulized salbutamol or ipratropium bromide) was additionally given. These therapies aimed to alleviate respiratory workload by reducing airway resistance.

Humidified oxygen therapy was administered via nasal cannula or oxygen mask when oxygen saturation fell below 92%. Nasal CPAP support was provided in cases of more severe hypoxemia and respiratory distress, and mechanical ventilation support was required in patients with severe and refractory respiratory distress. In complications due to viral infections, broad-spectrum antibiotic treatment was preferred if bacterial superinfection was suspected. Ampicillin-sulbactam or cephalosporin derivatives have been widely used in these cases. In cases accompanied by fever and markedly elevated CRP and WBC counts, antibiotic therapy was administered with caution. Hydration was considered an important part of treatment. Intravenous fluid support was provided to patients whose oral intake was not sufficient. The effects of immunoprophylactic treatments such as palivizumab have been studied in cases of severe RSV infection. However, such specific antiviral agents were only used in very high-risk patients.

STATISTICAL ANALYSIS

Data were analyzed using SPSS 22.0 software. Normal distribution of continuous variables was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. Analysis of variance test was used for group comparisons for normally distributed data, and Kruskal-Wallis test was used for non-normally distributed data. In multiple group comparisons, Bon-

ferroni correction was applied to control the Type I error rate (adjusted significance level=0.05/number of comparisons). Categorical variables were analyzed by chi-square test. $p<0.05$ was considered statistically significant. Statistical analyses were adjusted for potential confounding factors such as age, gender, chronic diseases and socioeconomic status. For this purpose, multivariate regression analyses were used and the results are presented adjusted for these factors.

ETHICAL CONSIDERATIONS

The study was approved by the Balıkesir Atatürk City Hospital Scientific Research Ethics Committee (date: September 19, 2024; no: 2024/09/52) and conducted in accordance with the 1964 Declaration of Helsinki. The identity information of the participants was kept confidential and data security was ensured. Since the study was conducted on the basis of retrospective data analysis, informed consent was not required.

RESULTS

STUDY POPULATION

The research participants received their diagnosis into 3 distinct categories: RSV positive (n=240, 60%) and influenza positive (n=100, 25%) and co-infection (n=60, 15%).

Demographic and Socioeconomic Characteristics

The mean age of patients diagnosed with acute bronchiolitis was 8.4±5.6 months. In gender distribution, 59% of the patients were male and 41% were female. Most patients (71%) resided in the city center, while 29% lived in rural areas. The summary of demographic and socioeconomic variables appears in Table 1. The study revealed that patients from low-income backgrounds experienced longer hospital stays (5.8±2.4 days vs. 4.2±1.9 days) and lower PNI scores (43.2±4.8 vs. 46.9±5.1) than patients from high-income families (p=0.032 and p=0.018 respectively).

TABLE 1: Demographic and clinical characteristics of patients with acute bronchiolitis (n=400)

Characteristics	n (%) or $\bar{X}$±SD
Age (months)	8.4±5.6
Gender	
Male	236 (59.0)
Female	164 (41.0)
Residence	
Urban	284 (71.0)
Rural	116 (29.0)
Maternal education level	
Primary school	124 (31.0)
Middle school	148 (37.0)
High school	88 (22.0)
University	40 (10.0)
Paternal education level	
Primary school	108 (27.0)
Middle school	156 (39.0)
High school	96 (24.0)
University	40 (10.0)
Monthly household income*	
Low (<minimum wage)	148 (37.0)
Moderate (1-2 times minimum wage)	188 (47.0)
High (>2 times minimum wage)	64 (16.0)

SD: Standard deviation

Socioeconomic status was categorized based on the minimum wage. Continuous variables are presented as mean±standard deviation, and categorical variables are presented as n (%). Chi-square test was used for gender comparison, and student's t-test was used for age comparison.

Seasonal Distribution of Viral Agents

Looking at the seasonal distribution of viral agents, RSV was found to be the most common agent throughout the year, peaking in December. RSV cases were also high in January and February, with a significant decrease in the summer months (June-August). Influenza cases were common during the winter months, with the highest incidence in December and January. Although co-infection cases were less frequent than RSV and influenza, these cases also increased in December and January. The incidence of all viral agents was minimal in the summer months and increased in the fall. These data reveal that acute bronchiolitis follows a distinct seasonal pattern during the winter months (Figure 1).

Laboratory Parameters

When laboratory parameters were analyzed, WBC, ANC, ALC and CRP levels were significantly higher in the co-infection group compared to the other groups. The mean WBC value was 13.6±5.4 $\times 10^3/\mu L$ in the co-infection group, significantly higher than RSV (12.4±5.1 $\times 10^3/\mu L$, p=0.042) and influenza groups (10.8±4.2 $\times 10^3/\mu L$, p=0.018). Similarly, ANC and ALC values were significantly different in the co-infection group. CRP levels were 18.2±14.6 mg/L

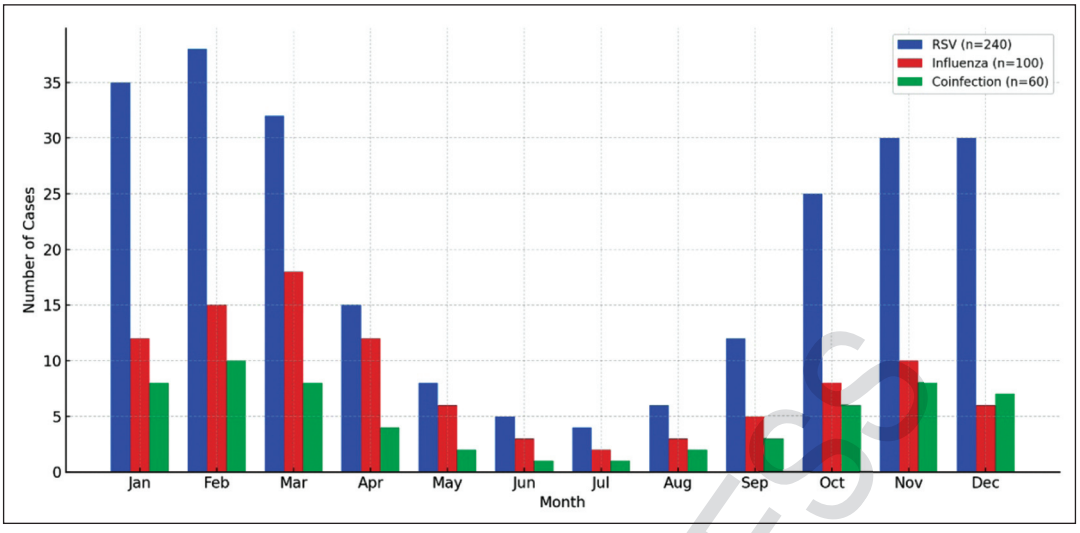

FIGURE 1: Monthly distribution of viral agents in acute bronchiolitis cases
RSV: Respiratory syncytial virus

in the co-infection group, compared to 15.8±13.2 mg/L in RSV (p=0.048) and 13.4±12.4 mg/L in influenza groups (p=0.024). However, no significant difference was found between the groups in terms of hemoglobin, hematocrit, platelet and biochemical parameters (Table 2).

Clinical Course and Hospitalization Periods

Hospitalization rates were highest in the co-infection group with 53.3%, compared to 35.0% in the RSV group and 18.0% in the influenza group (p=0.008). The rate of intensive care unit hospitalization was

TABLE 2: Laboratory findings of patients with acute bronchiolitis

Laboratory parameters	All patients (n=400)	RSV (+) (n=240)	Influenza (+) (n=100)	Co-infection (n=60)	p value*
Complete blood count					
WBC (×10³/μL)	11.2±4.8	12.4±5.1	10.8±4.2	13.6±5.4	0.024[a]
Hemoglobin (g/dL)	11.8±1.4	11.6±1.3	11.9±1.5	11.5±1.4	0.346[b]
Hematocrit (%)	35.4±3.8	35.2±3.6	35.6±3.9	35.0±3.7	0.528[b]
ANC (×10³/μL)	4.8±2.9	5.2±3.1	4.4±2.6	5.6±3.2	0.032[a]
ALC (×10³/μL)	4.2±2.1	4.6±2.3	4.0±1.9	4.8±2.4	0.038[a]
Platelet Count (×10³/μL)	342±112	338±108	345±115	336±110	0.842[b]
Biochemistry					
AST (U/L)	38.4±18.2	39.2±19.1	37.8±17.6	40.2±19.4	0.624[b]
ALT (U/L)	28.6±14.8	29.1±15.2	28.2±14.4	29.8±15.6	0.748[b]
BUN (mg/dL)	12.4±4.6	12.2±4.4	12.6±4.8	12.0±4.3	0.856[b]
Creatinine (mg/dL)	0.42±0.12	0.41±0.11	0.43±0.13	0.40±0.10	0.792[b]
Na (mEq/L)	138±3.2	137±3.0	139±3.4	136±2.8	0.246[b]
K (mEq/L)	4.2±0.6	4.1±0.5	4.3±0.7	4.0±0.4	0.342[a]
Inflammatory marker					
CRP (mg/L)	14.6±12.8	15.8±13.2	13.4±12.4	18.2±14.6	0.016[a]

*p values indicate intergroup comparisons; [a]p<0.05 is statistically significant; p>0.05 is not statistically significant; RSV: Respiratory syncytial virus; WBC: White blood cell count; ANC: Absolute neutrophil count; ALC: Absolute lymphocyte count; AST: Aspartate transaminase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen; CRP: C-reactive protein; ANOVA: Analysis of variance

Values are presented as mean±standard deviation. Groups were compared using ANOVA or Kruskal-Wallis test with Bonferroni correction. WBC, ANC, ALC, and CRP levels were higher in the co-infection group compared to the other groups

13.3% and the need for CPAP was 20.0% in the co-infection group, which were significantly higher than the other groups (p=0.006 and p=0.004).

When the duration of hospitalization was analyzed, the ward stay (referring to general pediatric ward admission) was 6.2±2.6 days and intensive care unit stay was 7.4±3.2 days in the co-infection group, both significantly longer than the other groups (p=0.018 and p=0.008). The mean duration of hospitalization was 4.8±2.2 days in the RSV group and 3.6±1.8 days in the influenza group. The rate of severe disease was highest in the co-infection group with 13.3%, while this rate was 5.0% in the RSV group and 2.0% in the influenza group (p=0.004) (Table 3).

Treatment Protocol Outcomes

The treatment plan for all patients included oxygen therapy and fluid control and respiratory assistance according to their needs. The co-infection group needed CPAP support at a rate of 32.1% whereas RSV patients needed it at 16.7% and influenza patients needed it at 12.0% (p=0.004). Mechanical ventilation was required in 14.3% of co-infection patients, 5.6% of RSV patients, and 4.0% of influenza patients (p=0.018). The total patient population received antibiotic treatment at 42.3% but the co-infection group received it at 56.7% compared to RSV at 40.8% and influenza at 35.0% (p=0.028).

Relationship Between PNI and Clinical Outcomes

The PNI showed that 46.7% (28/60) of patients with co-infection received poor PNI scores below 45 while the RSV group had 30.0% (72/240) and the influenza group had 25.0% (25/100) poor scores (p=0.006). While 46.7% of the patients in the co-infection group had a PNI score below 45, this rate was 30% in the RSV group and 25% in the influenza group. Among patients with poor PNI scores, the mean PNI value was lowest in the co-infection group (39.6±3.2) compared to RSV (41.2±2.8) and influenza groups (41.8±2.4) (p=0.012). The clinical course was more severe in patients with low PNI values. Especially in the co-infection group, the hospitalization rate reached 71.4% in patients with a PNI value below 45, while the average duration of hospitalization was 7.4±2.8 days. In these patients, the need for intensive care unit was 21.4% and the need for CPAP was 32.1%. The RSV group with low PNI showed a 50% hospitalization rate and patients spent an average of 5.8±2.6 days in the hospital. The influenza group with poor PNI had a 32% hospitalization rate and patients required 4.2±1.6 days of hospital care (p=0.004

TABLE 3: Relationship between viral agents and clinical course in patients with acute bronchiolitis (n=400)

Characteristics	RSV (+) (n=240)	Influenza (+) (n=100)	Co-infection (n=60)	p value*
Clinical course				
Outpatient treatment	156 (65.0)	82 (82.0)	28 (46.7)	0.012[a]
Hospitalization	84 (35.0)	18 (18.0)	32 (53.3)	0.008[a]
Ward admission	72 (30.0)	16 (16.0)	24 (40.0)	0.014[a]
ICU admission	12 (5.0)	2 (2.0)	8 (13.3)	0.006[a]
Length of stay (days)				
Ward	4.8±2.2	3.6±1.8	6.2±2.6	0.018[a]
ICU	5.6±2.4	4.2±2.0	7.4±3.2	0.008[a]
Respiratory support				
CPAP requirement	18 (7.5)	4 (4.0)	12 (20.0)	0.004[a]
Mechanical ventilation	6 (2.5)	1 (1.0)	5 (8.3)	0.002[a]
Disease severity				
Mild	156 (65.0)	82 (82.0)	28 (46.7)	0.008[a]
Moderate	72 (30.0)	16 (16.0)	24 (40.0)	0.012[a]
Severe	12 (5.0)	2 (2.0)	8 (13.3)	0.004[a]

*p values indicate intergroup comparisons; [a]p<0.05 is considered significant; RSV: Respiratory syncytial virus; ICU: Intensive care unit; CPAP: Continuous positive airway pressure
Categorical variables are presented as n (%), and continuous variables are expressed as mean±standard deviation. Chi-square test was used for categorical variables, and Kruskal-Wallis test was applied for length of stay. Disease severity was classified based on clinical findings and hospitalization requirements.

and p=0.008). The clinical course was better in patients with normal PNI values ≥45 in all viral agent groups; hospitalization in patients with PNI ≥45 in the RSV group was 28.6% and 13.3% in the influenza group, and 37.5% in the co-infection group. The need for intensive care and CPAP were also significantly lower in these patients. In all groups, the duration of hospitalization, need for intensive care and respiratory support were found to be statistically significantly higher in patients with poor PNI values (Table 4).

TABLE 4: Relationship between poor PNI score and clinical outcomes in viral agent groups

Characteristics	RSV (+)	Influenza (+)	Co-infection	p value*
Poor PNI (<45) patients				
Number of patients n (%)	72/240 (30.0)	25/100 (25.0)	28/60 (46.7)	0.006[a]
Mean PNI score	41.2±2.8	41.8±2.4	39.6±3.2	0.012[a]
Clinical outcomes in poor PNI group				
Hospitalization n (%)	36 (50.0)	8 (32.0)	20 (71.4)	0.004[a]
Length of hospital stay (days)	5.8±2.6	4.2±1.6	7.4±2.8	0.008[a]
ICU admission n (%)	8 (11.1)	2 (8.0)	6 (21.4)	0.006[a]
CPAP requirement n (%)	12 (16.7)	3 (12.0)	9 (32.1)	0.004[a]
Mechanical ventilation n (%)	4 (5.6)	1 (4.0)	4 (14.3)	0.018[a]
Mortality n (%)	0 (0.0)	0 (0.0)	1 (3.6)	0.042[a]
Normal PNI (≥45) patients				
Number of patients n (%)	168/240 (70.0)	75/100 (75.0)	32/60 (53.3)	0.006[a]
Mean PNI score	49.6±3.1	50.2±2.9	48.8±3.4	0.024[a]
Clinical outcomes in normal PNI group				
Hospitalization n (%)	48 (28.6)	10 (13.3)	12 (37.5)	0.012[a]
Length of hospital stay (days)	3.2±1.4	2.8±1.2	4.6±1.8	0.016[a]
ICU admission n (%)	4 (2.4)	0 (0.0)	2 (6.3)	0.024[a]
CPAP requirement n (%)	6 (3.6)	1 (1.3)	3 (9.4)	0.018[a]

*p values indicate intergroup comparisons between viral agent groups; [a]p<0.05 is considered statistically significant; RSV: Respiratory syncytial virus; PNI: Prognostic Nutritional Index; ICU: Intensive care unit; CPAP: Continuous positive airway pressure; ANOVA: Analysis of variance

Data are presented as n (%) or mean±standard deviation. Percentages for clinical outcomes are calculated based on the number of patients in each PNI subgroup.

Statistical analyses: Chi-square test for categorical variables, ANOVA or Kruskal-Wallis test for continuous variables.

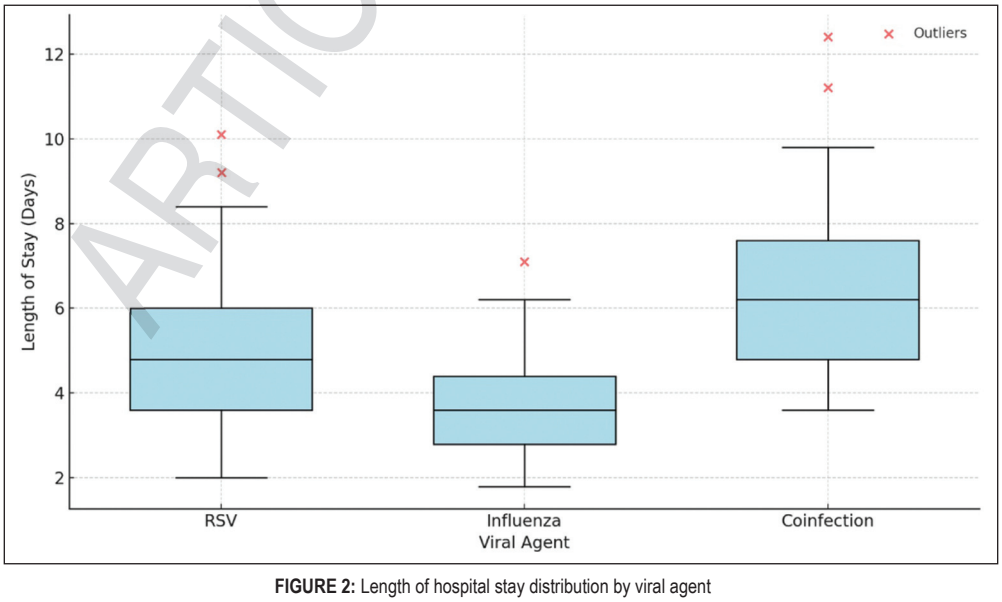

FIGURE 2: Length of hospital stay distribution by viral agent

RSV: Respiratory syncytial virus

Distribution of Length of Hospitalization

The mean length of hospitalization was 4.8±2.2 days in RSV positive patients, 3.6±1.8 days in influenza positive patients and 6.2±2.6 days in the co-infection group (p=0.018). In the co-infection group, it was noteworthy that outlier values were higher in addition to median values. In terms of the duration of intensive care unit hospitalization, the longest durations were found in the co-infection group (Figure 2).

DISCUSSION

In this study, we investigated RSV and influenza co-infection in 400 pediatric bronchiolitis patients. The main findings were: RSV predominance (60%), higher influenza rates than expected (25%), 15% co-infection rate, worse clinical outcomes in co-infected patients with poor PNI scores (<45), with 71.4% hospitalization rate and 7.4 days mean hospital stay in this subgroup.

The RSV rate detected in our study (60%) is consistent with the range of 50-80% reported in the international literature.[12] RSV is the most common viral cause of acute lower respiratory tract infections in children, especially in developing countries, with infection rates ranging from 20% to 75% in certain regions.[13,14] The dominant role of RSV can be explained by its tropism to bronchial epithelial cells, strong inflammatory response, cytokine storm, and impaired mucociliary activity.[15-18] The rate of influenza positivity (25%) is higher than the rates generally reported as 10-15% in the literature. Changes in the post-pandemic period and regional factors may explain this difference.[19,20] For example, Sánchez-Yebra et al. reported RSV responsible for 36.7% of lower respiratory tract infections.[21] In another post-pandemic study, RSV positivity rates increased by 3.2%, 6.6%, and 13.7% between 2020 and 2023, respectively, suggesting a link with sociodemographic and regional factors.[22]

The co-infection group displayed elevated inflammatory markers in laboratory tests with CRP levels reaching 18.2±14.6 mg/L compared to RSV (15.8±13.2 mg/L) and influenza groups (13.4±12.4 mg/L). This finding is consistent with previous studies showing that CRP levels are associated with disease severity in bronchiolitis.[23] The elevated WBC (13.6±5.4 $\times 10^3/\mu L$), ANC, and ALC in co-infected patients indicate enhanced inflammatory response. The high inflammatory response observed in the co-infection group may be associated with overactivation of neutrophils and inflammatory cytokine production in RSV infection. Neutrophils elevated WBC, CRP, and other inflammation markers, suggesting they contribute to aggravation of inflammation through activities such as NETosis and production of reactive oxygen species.[17] Babawale and Guerrero-Plata conducted research which showed that multiple viral infections lead to elevated proinflammatory cytokine production and worsened disease outcomes.[24] Inflammatory markers such as interleukin (IL)-8, IL-12, and IL-18 markedly potentiate neutrophil activity and inflammatory response.[25]

PNI and clinical outcomes the prognostic value of PNI score emerged as a significant finding in our study. The mean PNI score was significantly lower in the co-infection group (44.1±5.6) compared to RSV (46.8±5.2) and influenza groups (47.2±4.8). In the co-infection group, 46.7% of patients had a PNI score below 45, with higher rates of hospitalization, prolonged hospital stays, and increased need for intensive care, emphasizing its prognostic importance. Research shows PNI using albumin levels and lymphocyte count effectively predicts both disease severity and treatment results for pediatric respiratory infections.[26,27] The hospitalization rate (71.4%) and mean duration of hospitalization (7.4 days) in patients with low PNI scores highlight the impact of nutritional status and immune response on disease severity.[10,28] The study by Wang et al demonstrated that PNI values below normal thresholds linked to longer intensive care unit hospitalization and increased mechanical ventilation requirements and elevated 30- and 90-day mortality rates in community-acquired pneumonia patients thus establishing its predictive value for severe respiratory infections. [26,27] The combination of inadequate nutrition and viral co-infection produces a combined effect that makes diseases more severe because our co-infected patients with low PNI scores needed CPAP at a rate of 32.1%.

In terms of the need for respiratory support, the use of b-CPAP in the early period reduces the need

for mechanical ventilation and prevents treatment failure. This emphasizes the importance of b-CPAP in bronchiolitis treatment.[29] Studies by Babawale and Guerrero-Plata show that RSV and human metapneumovirus co-infections can cause severe airway pathology, leading to mechanical ventilation.[24] The need for increased respiratory support is associated with severe bronchial inflammation, increased mucus secretion, and airway resistance.

The seasonal distribution observed overlaps with patterns reported in the literature. RSV and influenza cases peak in winter months due to low temperatures and humidity, which facilitate viral replication and suppress the immune system.[6] RSV and influenza co-infection cases also follow this seasonal pattern, likely due to similar environmental factors.[10]

The study showed that patients from low-income backgrounds needed longer hospital stays and their PNI scores were lower which we included in the results section. Urban risk factors such as crowded living conditions play an important role in bronchiolitis. In the study by Jansson et al., children of low socioeconomic status were more frequently hospitalized.[29]

Our research had limitations: the single-center design limits generalizability, some clinical and laboratory data were missing due to the study's retrospective nature, and follow-up results could not be assessed. Viral agents other than RSV and influenza were not analyzed, potentially overlooking other co-infections. The study failed to obtain data about smoking exposure and breastfeeding status which could affect both the severity of bronchiolitis and its treatment results. The study faces a limitation because it does not contain data about vaccination status including influenza vaccination. However, the strengths of our study include the appropriate sample size, complex laboratory data, and the ability to estimate seasonal distribution over two years. Including sociodemographic data helped identify bronchiolitis risk factors, while examining ward and intensive care unit processes provided a comprehensive analysis.

CONCLUSION

RSV and influenza co-infection in patients with poor nutritional status (PNI <45) needs immediate detection and intensive treatment because it creates a dangerous medical situation. The PNI score needs to become a standard tool for assessing bronchiolitis patients because it helps doctors predict which patients will experience severe complications. The combination of early nutritional care with continuous observation of patients with low PNI scores who have co-infections leads to better medical results. Healthcare providers need to keep a strong alert for co-infections throughout winter months while providing prompt respiratory care to patients in this vulnerable group. Researchers should conduct future studies to confirm specific PNI thresholds for pediatric bronchiolitis and assess the effectiveness of individualized nutritional care plans.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Özlem Özcanlı Çay, Özlem Kemer Aycan;* ***Design:*** *Özlem Özcanlı Çay;* ***Control/Supervision:*** *Özlem Özcanlı Çay, Özlem Kemer Aycan;* ***Data Collection and/or Processing:*** *Özlem Özcanlı Çay;* ***Analysis and/or Interpretation:*** *Özlem Özcanlı Çay, Özlem Kemer Aycan;* ***Literature Review:*** *Özlem Özcanlı Çay, Özlem Kemer Aycan;* ***Writing the Article:*** *Özlem Özcanlı Çay, Özlem Kemer Aycan;* ***Critical Review:*** *Özlem Kemer Aycan;* ***References and Fundings:*** *Özlem Özcanlı Çay.*

REFERENCES

1. Florin TA, Plint AC, Zorc JJ. Viral bronchiolitis. Lancet. 2017;389(10065):211-24. PMID: 27549684; PMCID: PMC6765220
2. Ghazaly MMH, Abu Faddan NH, Raafat DM, Mohammed NA, Nadel S. Acute viral bronchiolitis as a cause of pediatric acute respiratory distress syndrome. Eur J Pediatr. 2021;180(4):1229-34. PMID: 33161501; PMCID: PMC7648537
3. Baraldi E, Lanari M, Manzoni P, Rossi GA, Vandini S, Rimini A, et al. Inter-society consensus document on treatment and prevention of bronchiolitis in newborns and infants. Ital J Pediatr. 2014;40:65. PMID: 25344148; PMCID: PMC4364570
4. Manti S, Staiano A, Orfeo L, Midulla F, Marseglia GL, Ghizzi C, et al. UPDATE - 2022 Italian guidelines on the management of bronchiolitis in infants. Ital J Pediatr. 2023;49(1):19. PMID: 36765418; PMCID: PMC9912214
5. Smith DK, Seales S, Budzik C. Respiratory syncytial virus bronchiolitis in children. Am Fam Physician. 2017;95(2):94-9. PMID: 28084708
6. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al; Respiratory Virus Global Epidemiology Network; Nair H; RESCEU investigators. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. Lancet. 2022;399(10340):2047-64. PMID: 35598608; PMCID: PMC7613574
7. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet. 2011;377(9773):1264-75. PMID: 21435708; PMCID: PMC7138033
8. Caballero MT, Polack FP, Stein RT. Viral bronchiolitis in young infants: new perspectives for management and treatment. J Pediatr (Rio J). 2017;93 Suppl 1:75-83. PMID: 28859915
9. Chen S, Wang Y, Li A, Jiang W, Xu Q, Wu M, et al. Etiologies of hospitalized acute bronchiolitis in children 2 years of age and younger: a 3 years' study during a Pertussis epidemic. Front Pediatr. 2021;9:621381. PMID: 34458206; PMCID: PMC8397516
10. Bermúdez-Barrezueta L, López-Casillas P, Rojo-Rello S, Sáez-García L, Marugán-Miguelsanz JM, de la Asunción Pino-Vázquez M. Outcomes of viral coinfections in infants hospitalized for acute bronchiolitis. Virol J. 2023;20:235. https://virologyj.biomedcentral.com/articles/10.1186/s12985-023-02197-7#citeas
11. Johns Hopkins All Children's Hospital: Bronchiolitis Clinical Pathway. St. Petersburg (FL): Johns Hopkins All Children's Hospital; 2020. https://www.hopkinsmedicine.org/-/media/files/allchildrens/clinical-pathways/bronchiolitis-pathway_-2_7_2020-ct.pdf
12. Ramagopal G, Brow E, Mannu A, Vasudevan J, Umadevi L. Demographic, clinical and hematological profile of children with bronchiolitis: a comparative study between respiratory synctial virus [RSV] and [Non RSV] groups. J Clin Diagn Res. 2016;10(8):SC05-8. PMID: 27656520; PMCID: PMC5028508
13. Naz R, Gul A, Javed U, Urooj A, Amin S, Fatima Z. Etiology of acute viral respiratory infections common in Pakistan: a review. Rev Med Virol. 2019;29(2):e2024. PMID: 30548740; PMCID: PMC7169323
14. Ali A, Akhund T, Warraich GJ, Aziz F, Rahman N, Umrani FA, et al. Respiratory viruses associated with severe pneumonia in children under 2 years old in a rural community in Pakistan. J Med Virol. 2016;88(11):1882-90. PMID: 27096404; PMCID: PMC7166621
15. Petat H, Gajdos V, Angoulvant F, Vidalain PO, Corbet S, Marguet C, et al. High frequency of viral co-detections in acute bronchiolitis. Viruses. 2021;13(6):990. PMID: 34073414; PMCID: PMC8229544
16. Binns E, Tuckerman J, Licciardi PV, Wurzel D. Respiratory syncytial virus, recurrent wheeze and asthma: a narrative review of pathophysiology, prevention and future directions. J Paediatr Child Health. 2022;58(10):1741-6. PMID: 36073299; PMCID: PMC9826513
17. Sebina I, Phipps S. The contribution of neutrophils to the pathogenesis of RSV bronchiolitis. Viruses. 2020;12(8):808. PMID: 32726921; PMCID: PMC7472258
18. Simpson J, Loh Z, Ullah MA, Lynch JP, Werder RB, Collinson N, et al. Respiratory syncytial virus infection promotes necroptosis and HMGB1 release by airway epithelial cells. Am J Respir Crit Care Med. 2020;201(11):1358-71. PMID: 32105156
19. Nair H, Brooks WA, Katz M, Roca A, Berkley JA, Madhi SA, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet. 2011;378(9807):1917-30. PMID: 22078723
20. Wang X, Li Y, O'Brien KL, Madhi SA, Widdowson MA, Byass P, et al; Respiratory Virus Global Epidemiology Network. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modelling study. Lancet Glob Health. 2020;8(4):e497-510. PMID: 32087815; PMCID: PMC7083228
21. Sánchez-Yebra W, Ávila-Carrillo JA, Giménez-Sánchez F, Reyes-Bertos A, Sánchez-Forte M, Morales-Torres M, et al; LRTI Research Group. Viral agents causing lower respiratory tract infections in hospitalized children: evaluation of the Speed-Oligo® RSV assay for the detection of respiratory syncytial virus. Eur J Clin Microbiol Infect Dis. 2012;31(3):243-50. PMID: 21647616; PMCID: PMC7088155
22. Korsun N, Trifonova I, Madzharova I, Alexiev I, Uzunova I, Ivanov I, et al. Resurgence of respiratory syncytial virus with dominance of RSV-B during the 2022-2023 season. Front Microbiol. 2024;15:1376389. PMID: 38628867; PMCID: PMC11019023
23. Jewell NA, Vaghefi N, Mertz SE, Akter P, Peebles RS Jr, Bakaletz LO, et al. Differential type I interferon induction by respiratory syncytial virus and influenza a virus in vivo. J Virol. 2007;81(18):9790-800. PMID: 17626092; PMCID: PMC2045394
24. Babawale PI, Guerrero-Plata A. Respiratory viral coinfections: insights into epidemiology, immune response, pathology, and clinical outcomes. Pathogens. 2024;13(4):316. PMID: 38668271; PMCID: PMC11053695
25. Bataki EL, Evans GS, Everard ML. Respiratory syncytial virus and neutrophil activation. Clin Exp Immunol. 2005;140(3):470-7. PMID: 15932508; PMCID: PMC1809401
26. Wang G, Wang N, Liu T, Ji W, Sun J, Lv L, et al. Association between prognostic nutritional index and mortality risk in patients with community-acquired pneumonia: a retrospective study. BMC Pulm Med. 2024;24(1):555. PMID: 39501259; PMCID: PMC11536940
27. De Rose L, Sorge J, Blackwell B, Benjamin M, Mohamed A, Roverts T, et al. Determining if the prognostic nutritional index can predict outcomes in community acquired bacterial pneumonia. Respir Med. 2024;226:107626. PMID: 38583813
28. Caballero MT, Polack FP, Stein RT. Viral bronchiolitis in young infants: new perspectives for management and treatment. J Pediatr (Rio J). 2017;93 Suppl 1:75-83. PMID: 28859915
29. Jansson L, Nilsson P, Olsson M. Socioeconomic environmental factors and hospitalization for acute bronchiolitis during infancy. Acta Paediatr. 2002;91(3):335-8. PMID: 12022309