

Clinical and epidemiologic factors related to subsequent wheezing after virus-induced lower respiratory tract infections in hospitalized pediatric patients younger than 3 years

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Abstract Most wheezing episodes in infants are caused and exacerbated by virus-induced lower respiratory tract infections. However, there are few reports of epidemiologic and clinical virus-specific research with a focus on virus-induced wheezing. The purpose of the current study was to characterize the clinical presentation of virus-induced wheezing in pediatric patients <3 years of age who were hospitalized with lower respiratory tract infections. Of the 412 patients in the study, 216 were followed for 3 years. Nasopharyngeal aspirates collected from the patients at the time of admission were examined for the presence of respiratory syncytial virus (RSV), rhinovirus (RV), parainfluenza-3 virus (PIV-3), human metapneumovirus (hMPV), and influenza virus (Flu) using reverse-transcription polymerase chain reaction and rapid diagnostic tests. Clinical signs were assessed using a severity scoring system. In patients with wheezing at the time of admission, RSV, RV, RSV+RV, Flu, PIV-3, and hMPV were detected in 33, 14, 8, 8, 5, and 3 % of samples, respectively. There were no differences in age and severity scores between patients harboring more prevalent viruses (RSV and RV) and those with less common infections. Patients with wheezing and RV-positive aspirates at the time of admission were more likely to develop subsequent wheezing during the following 3 years. **Conclusion:** RSV and RV infections are factors in the development and exacerbation of wheezing after virus-induced lower respiratory tract infections. Moreover, RV-

induced wheezing may be associated with subsequent recurrent wheezing and the development of asthma.

Keywords Virus-induced wheezing · Subsequent wheezing · 3-year follow-up · Lower respiratory tract infection

Introduction

Most wheezing in infants is caused and exacerbated by lower respiratory tract infections [40]. Viruses that cause lower respiratory tract infections associated with wheezing include respiratory syncytial virus (RSV), rhinovirus (RV), human metapneumovirus (hMPV), influenza virus (Flu), parainfluenza virus (PIV), and human bocavirus (hBoV) [23, 40].

Infants hospitalized with lower respiratory tract infections and wheezing are at significantly increased risk for recurrent wheezing and childhood asthma [36]. RSV is the main indication for hospitalization during the first wheezing episode in infants, and RSV appears to be the pathogen associated with recurrent wheezing [1, 5]. Recent studies have shown that RV is more likely to be associated with recurrent wheezing and the development of asthma after lower respiratory tract infections and wheezing than RSV [20, 31].

In the current study, respiratory viruses were detected, using the polymerase chain reaction (PCR) and a rapid diagnostic kit, in nasopharyngeal aspirates collected from patients hospitalized with lower respiratory tract infections. The difference in the frequency of wheezing and the clinical presentation of patients infected with respiratory viruses was determined. If children with RV and RSV infections presented with wheezing at the time of admission, the development of subsequent wheezing was associated with clinical severity, age, viral load, and a history of allergies.

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Materials and methods

Patients

Nasopharyngeal aspirates were collected between February 2008 and August 2009 from 412 pediatric patients ≤ 3 years of age who were hospitalized for lower respiratory tract infections at the Soma General Hospital, in Soma City, which is located in the northeastern part of Fukushima prefecture, Japan. The Soma General Hospital is the only hospital in the area that admits children. The study was approved by the Ethical Committee of the Soma General Hospital, and informed consent for participation in the study was obtained from the parents of each patient.

The disease severity of each patient was estimated and scored according to a severity scoring system, as described previously (Table 1) [17, 28]. Blood leukocyte counts, blood eosinophil counts, and total serum immunoglobulin (Ig)E concentrations were measured at the time of enrollment. Blood eosinophils ≥ 5 % of the whole leukocyte counts and a total serum IgE ≥ 30 IU/ml (standard value for children ≤ 3 years of age) were regarded as elevated. IgE antibody titers to common allergens were determined using a fluoroenzyme immunoassay (cut-off level = 0.35 kU/l for *Dermatophagoides pteronyssinus*, house dust, egg, cow's milk, peanuts, wheat, soybean, buckwheat, rice, sardine, gelatin, mold, and animal epithelium; fluoroenzyme immunoassay, CAP allergen: Bio Medical Laboratories, Inc., Tokyo, Japan).

Virus detection

Nasopharyngeal aspirates were treated as described by Drosten et al. [10]. Briefly, the nasopharyngeal samples were

weighed, gently shaken for 30 min with an equal volume of acetylcysteine (10 g/l) in 0.9 % sodium chloride, and centrifuged for 10 min. The supernatants were stored at -80°C until analysis.

Viral RNA was extracted from 200 μl of specimen supernatants using a QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany), cDNA was synthesized using a PrimeScript RT Reagent Kit (Takara Bio Inc., Shiga, Japan) and an ABI 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA), according to the manufacturers' instructions.

Four methods were used for viral detection: Real-time (quantitative) reverse-transcription (RT) PCR (qRT-PCR): RSV [19] and RV [34]; RT PCR: PIV-3 [41]; conventional PCR: hMPV [11]; and a rapid diagnostic test (Capillia Flu A+B; Alfresa Pharma Corporation, Tokyo, Japan): Influenza A and B. The RV primer pair and probe were designed to detect all RVs described previously [34], including RV-C, but not to differentiate between species. RSV and RV copy numbers were determined using a serially diluted plasmid standard of known concentration. The copy number of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured in each sample and used for sample normalization.

Follow-up

Detailed demographic, clinical, and laboratory data were obtained from the parents of the patients with a structured questionnaire and from patient medical files. The studied variables included a history of wheezing and atopic disease, a family history of asthma and atopic disease, the presence of siblings, family smoking habits, pets in the home, and daycare attendance.

Approximately 3 years after discharge, the information on subsequent wheezing was obtained from all patient medical files. The term "subsequent wheezing" was defined as a visit to the Soma General Hospital or a local medical clinic with a wheezing episode, and/or requiring long-term therapy for physician-diagnosed asthma, and/or hospitalization with an asthma attack. The patients who did not visit the Soma General Hospital after discharge or whose detailed demographic, clinical, and previous data at the time of admission were not obtained were excluded from analyses.

Statistical analysis

The descriptive analysis included calculated percentages, median and range for demographic variables, and laboratory data. One-way analysis of variance was used to examine the differences in viruses. Differences between groups were tested for significance using a non-parametric Mann-Whitney test for non-normally distributed data. A chi-square test was used to

Table 1 Severity scoring system

Symptom	Points
Fever ($\geq 38^{\circ}\text{C}$)	1
Cough	
Mild	1
Moderate	2
Severe	3
Rhinorrhea	
Mild ^a	1
Moderate to severe ^b	2
Hoarseness	1
Duration of illness >4 days	1
Apnea	3
Wheezing	5
Cyanosis	5
Retractions	5
Tachypnea	5

^a Suction 0–4 times/day or wipe every 2 h or less

^b Suction ≥ 5 times/day or ≥ 1 wipe/h

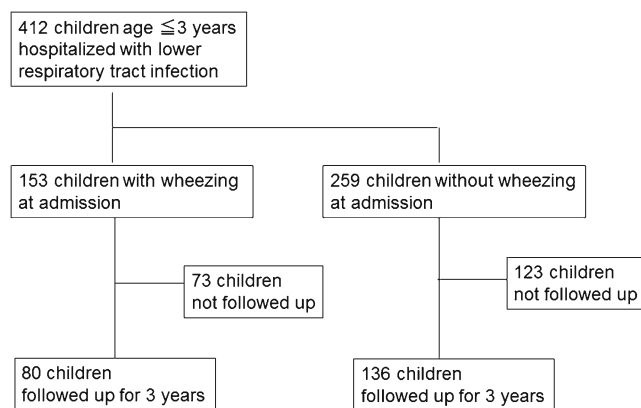


Fig. 1 Study flowchart

qualitatively analyze categorical independent variables. *P* values ≤ 0.05 were considered statistically significant. Analyses were performed using the Statistical Package for Social Sciences (version 17.0; SPSS, Inc., Chicago, IL, USA).

Results

During the study period, 412 pediatric patients (≤ 3 years of age) hospitalized with lower respiratory tract infections were enrolled in this study. The patients were divided into two groups: the wheezing at admission group ($n=153$) and the no wheezing at admission group ($n=259$). Follow-up examinations were performed with 52 % of members from each group: 80 (52 %) patients in the wheezing at admission and 136 (52 %) in the no wheezing at admission group (Fig. 1).

Viruses were detected in the nasal aspirates from 235 of the 412 patients (57 %). RSV, RV, RSV+RV, Flu, PIV-3, and hMPV were detected in 114 (28 %), 43 (11 %), 22 (6 %), 21 (5 %), 16 (4 %), and 8 (2 %) patients, respectively. Five other co-infections were detected in 11 patients (RSV+Flu ($n=3$), RSV+hMPV ($n=2$), RV+Flu ($n=3$), RV+hMPV ($n=1$), PIV-3+hMPV ($n=1$)). Because the 11 patients with these co-infections were in the minority, they were excluded from this study.

Of the 153 patients with wheezing at the time of admission, viruses were found in 102 (67 %) samples: RSV, RV, RSV+

RV, Flu, PIV-3, and hMPV were detected in 51 (33 %), 21 (14 %), 12 (8 %), 5 (3 %), 8 (4 %), and 5 (2 %) patients, respectively (Fig. 2a). Of the 259 patients without wheezing at the time of admission, viruses were found in 122 (47 %) samples: RSV, RV, RSV+RV, Flu, PIV-3, and hMPV were detected in 63 (25 %), 22 (9 %), 10 (4 %), 16 (7 %), 8 (3 %), and 3 (1 %) patients, respectively. Viruses were not found in 126 (51 %; Fig. 2b) of non-wheezing patients. Viruses were detected in a higher percentage of patients with wheezing at the time of admission than in patients without wheezing.

The 153 patients with wheezing at the time of admission were divided into two age groups (≤ 12 months ($n=67$) and 1–3 years ($n=86$)), and the virus detection results were compared as a function of age (Fig. 3). Of the 67 patients ≤ 12 months of age, RSV, RV, and RSV+RV were detected in 24, 8, and 10 patients, respectively; thus, RV and/or RSV were detected in 42 patients. Of the 86 patients 1–3 years of age, RSV and/or RV were detected in 42 patients. RSV and RV were detected more often in the children ≤ 12 months of age than the children 1–3 years of age.

The number of patients presenting with wheezing at the time of admission, the age at the time of admission, and the severity scores were compared among the viruses detected (Table 2). The number of virus-positive patients who presented with wheezing was as follows: RSV, 51; RV, 21; RSV+RV, 12; Flu, 5; PIV-3, 8; and hMPV 5 and unknown, 51. Patients in whom RSV, RV, RSV+RV, and hMPV were detected were more often associated with wheezing at the time of admission than the unknown group. The median ages at the time of admission for each virus were as follows: RSV, 13 months; RV, 13 months; RSV+RV, 6 months; Flu, 15 months; PIV-3, 15 months; hMPV, 9 months; and unknown, 13 months. There were no significant differences in age at the time of admission for any of the viruses. The median severity scores for each virus were as follows: RSV, 10; RV, 14; RSV+RV, 13; Flu, 7; PIV-3, 9.5; hMPV, 10; and unknown, 9. The severity scores for RV or RSV+RV were significantly higher than the scores for the Flu or unknown group. There were no significant differences in age at the time of admission and severity scores between the RSV- and RV- detected patients.

Two hundred sixteen patients were followed for 3 years: 80 patients in the wheezing at admission group and 136 patients

Fig. 2 Viruses detected in the presence or absence of wheezing at admission. **a** Viruses detected in children with wheezing at admission ($n=153$). **b** Viruses detected in children without wheezing at admission ($n=259$)

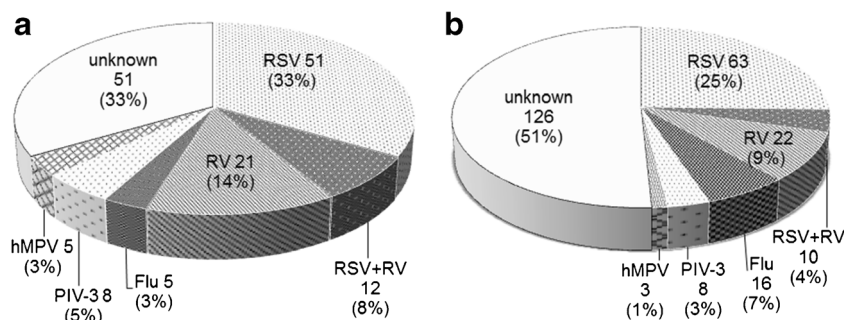
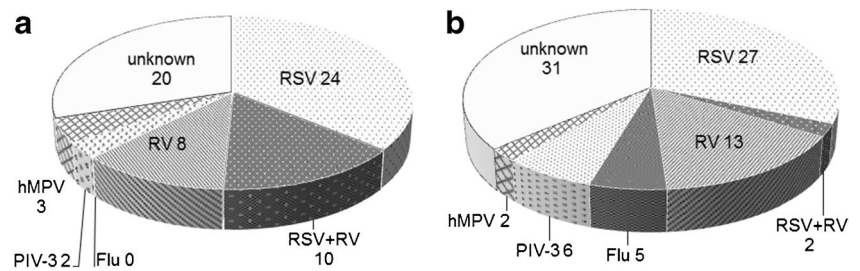


Fig. 3 Age distribution of viral infections among patients with wheezing at admission. **a** Viruses detected in patients aged <12 months ($n=67$). **b** Viruses detected in patients aged 1–3 years ($n=86$)



in the no wheezing at admission group. The patients' numbers or median and range of the demographic and clinical factors in each group are shown in Table 3. Differences between groups were tested for significance using a Mann-Whitney test or a chi-square test. There were significant differences in the age at the time of hospitalization, severity score, days of hospitalization in both groups, gestational age, a history of wheezing, atopic disease of siblings, daycare attendance, and the blood eosinophil count at admission. The patients with wheezing at admission were younger age, had higher disease severity, more history of previous wheezing, and higher blood eosinophil count than patients without wheezing at admission.

The frequency of subsequent wheezing in the wheezing and no wheezing at admission groups and the detected viruses are shown in Fig. 4. In the patients with wheezing at admission, 9 of 11 patients in whom RV was detected presented with wheezing during the 3-year follow-up, and 11 of 25 patients in

whom RSV was detected presented with wheezing during the 3-year follow-up. Patients in whom RV was detected presented with wheezing more often than patients in whom RSV was detected.

Wheezing at admission, age, severity score, viral load, allergic predisposition, and subsequent wheezing were assessed in patients in whom RSV or RV was detected (Table 4). Allergic predisposition was defined as high IgE antibody levels (>30 IU/ml) at the time of admission and/or a parental history of asthma. In patients in whom RV was detected, those with subsequent wheezing exhibited more wheezing at the time of admission and higher severity scores than patients with no subsequent wheezing. Patients in whom RV and RSV were detected showed no significant difference in age at admission and viral load between the subsequent wheezing and no wheezing groups. Subsequent wheezing in the RV group was associated with wheeze on admission and a

Table 2 Virus distribution and clinical characteristics of 153 pediatric patients with wheezing at admission

Patients with wheezing			Age (months) (median and range)	Severity score (median and range)
RSV ($n=114$)	51	*	13 (3–47)	10 (8–24)
RV ($n=43$)	21	**	13 (1–40)	14 (8–23)
RSV+RV ($n=22$)	12	***	6 (1–40)	13 (8–25)
Flu ($n=21$)	5		20 (12–46)	7 (7–8)
PIV-3 ($n=16$)	8		15 (6–44)	9.5 (9–14)
hMPV ($n=8$)	5	****	9 (3–18)	10 (9–15)
Unknown ($n=177$)	51		13 (2–41)	9 (8–15)

One-way analysis of variance

* $p=0.005$

** $p=0.012$

*** $p=0.014$

**** $p=0.043$

***** $p=0.000$

***** $p=0.007$

***** $p=0.036$

Table 3 Demographic and clinical factors of patients followed for 3 years

	Wheezing at admission (<i>n</i> =80)	Without wheezing at admission (<i>n</i> =136)	
Age at hospitalization (months)	13 (1–47) ^a	15 (0–47) ^a	0.006 ^c
Male	50	74	0.246 ^d
Severity score (points)	9.5 (7–28) ^a	4 (2–10) ^a	0.000 ^c
Days of hospitalization	5 (3–14) ^a	5 (2–11) ^a	0.003 ^c
Gestational age (weeks)	38 (28–42) ^a	39 (29–42) ^a	0.017 ^c
Birth body weight (g)	2,952 (692–3,940) ^a	2,985 (522–3,972) ^a	0.106 ^c
Premature and/or low birth-weight	16	15	0.069 ^d
Perinatal abnormality	5	2	0.055 ^d
Previous history of wheezing	54	62	0.002 ^d
Atopic dermatitis	16	15	0.149 ^d
Food allergy	7	13	0.843 ^d
Family history of asthma	24	31	0.240 ^d
Parental asthma	15	19	0.352 ^d
Asthma of siblings	16	17	0.139 ^d
Family history of atopic disease other than asthma	35	58	0.874 ^d
Parental atopic disease other than asthma	27	52	0.509 ^d
Atopic diseases of siblings other than asthma	11	7	0.027 ^d
Siblings	53	84	0.509 ^d
Pets in home	23	55	0.084 ^d
Environmental tobacco smoke	59	96	0.618 ^d
Daycare attendance	35	79	0.042 ^d
Blood eosinophils at admission (%)	1 (0–26) ^a	0 (0–23) ^a	0.049 ^e
Blood leukocyte cells at admission (/μl)	12,700 (2,800–25,700) ^a	10,900 (2,900–36,900) ^a	0.215 ^e
IgE antibody (IU/ml)	12.45 (5–5,540) ^{a,b}	24.6 (5–2,240) ^{a,c}	0.225 ^e
IgE antibody >30 IU/ml ^c	28 ^b	52 ^c	0.634 ^d
Positive response to any allergen ^c	27 ^b	41 ^c	0.582 ^d
Positive response to aeroallergen ^c	18 ^b	28 ^c	0.740 ^d
Positive response to food allergen ^c	18 ^b	23 ^c	0.312 ^d

^a Median and range^b *n*=72^c *n*=122^d Chi-square test^e Mann-Whitney test

high severity score. Subsequent wheezing in the RSV group was associated with allergic predisposition.

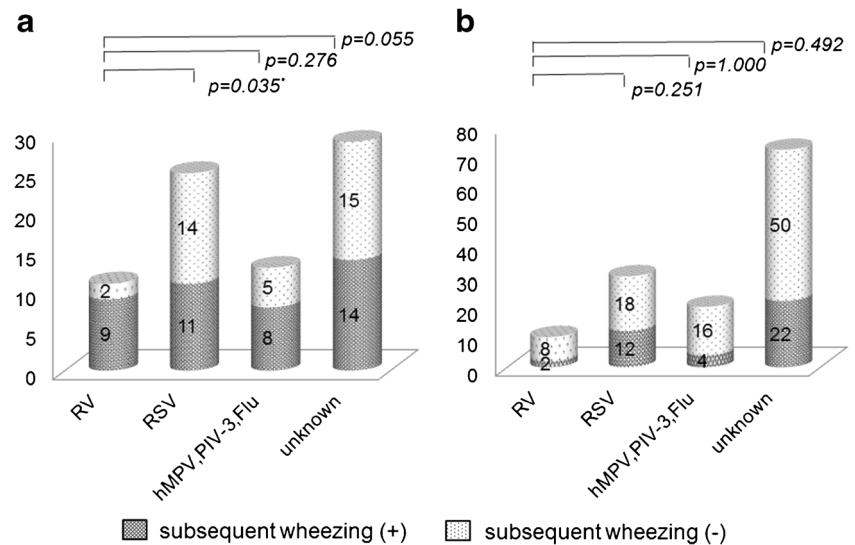
Discussion

There are few reports of epidemiological and clinical virus-specific research with a focus on virus-induced wheezing. Fujitsuka et al. [13] reported that viruses were detected in 86.1 % of 115 Japanese children with wheezing (mean age, 20.8 months): specifically, RSV (40.9 %), RV (31.3 %), RSV+RV (12.2 %), hMPV (0.9 %), and PIV (0.9 %). García-García et al. [15] reported that a viral pathogen was identified in 71 % of children <14 years of age who were admitted to the hospital with an acute wheezing episode: RSV (27 %), RV (24 %), adenovirus (17.8 %), human bocavirus (hBoV; 16 %), and hMPV (4.7 %). The rate of viral detection was higher in infants (77.3 %) than in older children (59.8 %),

and RSV and hBoV were more prevalent in infants than in older children. In the current study, the rate of viral detection was 67 % in pediatric patients ≤3 years of age with wheezing who were hospitalized for lower respiratory tract infections, lower than the previously cited studies. This lower detection rate may be because hBoV and adenovirus were not targeted for detection. In the current study, RSV and RV were detected more frequently in patients who had wheezing and in infants in particular, than in patients without wheezing. Thus, RSV and RV were more frequently associated with wheezing in infants.

RSV and RV were detected in 8 % of patients with acute wheezing, with a higher rate of detection in the group ≤12 months of age than in the group 1–3 years of age. Fujitsuka et al. [13] reported that RSV and RV were detected in 12.2 % of Japanese children with acute wheezing illnesses, and Chung et al. [8] detected RSV and RV in 3.9 % of Korean children with acute wheezing illnesses: neither study carried

Fig. 4 The number of patients with subsequent wheezing after viral infection with or without wheezing at the time of admission. **a** Patients with wheezing at admission. **b** Patients without wheezing at admission. *3-year follow-up (chi-square test)



out classification by age. In the study, the basis for the differences in the detection rate of RSV+RV between the groups ≤ 12 months of age and 1–3 years of age was not determined.

A number of studies involving RSV-induced recurrent wheezing have shown that approximately one half of infants with RSV bronchiolitis progress to recurrent wheezing during childhood [1, 3, 45]. In the 1980s and 1990s, it was thought that RSV-specific IgE responses in infants with bronchiolitis were related to recurrent wheezing [33, 42, 43]. It has been proposed that RSV infection stimulates type 2 cytokine responses, such as Interleukin (IL)-4; IL-10; and RANTES, which may induce a Th2-dominant immune state, modify the original allergic predisposition of patients, and therefore induce recurrent wheezing or the development of asthma [2, 4, 9, 12, 32, 35]. In the current study, patients with an allergic predisposition in whom RSV was detected presented more often with subsequent wheezing. However, the mechanisms

underlying the association between RSV infection and long-term airway morbidity is poorly understood.

Recently, many studies have suggested that bronchiolitis caused by viruses other than RSV leads to recurrent wheezing [7, 14, 38]. Specifically, it has been reported that RV is associated with severe lower respiratory tract infections [29, 37, 39], and RV-associated early wheezing may be a significant risk factor for the development of recurrent wheezing [16, 24, 27]. Indeed, RV may pose a greater risk of recurrent wheezing and development of asthma than RSV [21, 26], and the genetic risk factor of asthma has been associated with RV wheezing in early life, but not with RSV wheezing [6]. The results of current study suggest that viral infection-induced severe lower respiratory tract infections are associated with subsequent wheezing because patients with wheezing at admission in whom RV was detected were more likely to have subsequent wheezing. In contrast, patients without wheezing

Table 4 Factors related to subsequent wheezing in pediatric patients with RV or RSV infection

	RV			RSV		
	Subsequent wheezing (+) (n=11)	Subsequent wheezing (-) (n=10)		Subsequent wheezing (+) (n=23)	Subsequent wheezing (-) (n=32)	
Patients with wheezing at admission	9	2	$p=0.005^b$	11	14	$p=0.765^b$
Age (months)	12 (1–28) ^a	11 (7–32)	$p=0.525^c$	14 (3–47)	13.5 (3–45)	$p=0.645^c$
Severity score (points)	18 (8–28)	5 (3–19)	$p=0.003^c$	9 (2–24)	5 (3–20)	$p=0.352^c$
Viral load (Log ₁₀ copies /ml)	4.3 (2.1–6.6)	4.2 (2.9–5.5)	$p=0.833^c$	1.3 (-2.0–5.4)	2.6 (-2.1–5.4)	$p=0.213^c$
Patients had high IgE (>30 IU/ml) and/or parental history of asthma	6	3	$p=0.387^b$	15 (n=21)	9 (n=30)	$p=0.005^b$

3-year follow-up

^a Median and range

^b Chi-square test

^c Mann-Whitney test

at admission in whom RV was detected were less frequently associated with subsequent wheezing in this study. Message et al. [30] demonstrated that RV infection induces more lower respiratory symptoms and lung function impairment, the RV load was strongly related to the severity of clinical illness in asthmatic patients, and Th2 cytokines were associated with more asthma symptoms in an experimental model of RV-induced asthma exacerbations. The current study and other reports suggest that the lower tract inflammation caused by RV infections increases bronchial hyperactivity and is associated with subsequent wheezing.

The current study showed that the factors related to subsequent wheezing are allergic predisposition in RSV and wheezing and clinical severity at admission in RV; viral load of RSV and RV in nasal aspirates at admission were not related to subsequent wheezing. The authors previously reported that there was a significant negative correlation between disease severity and age in infants and a significant positive correlation between disease severity and viral load in older children with RV infections [37]. Thus, in children with RV infections, it is suggested that both viral load and age, but not viral load only or age alone, are associated with disease severity at the time of admission, and severe RV infection may be associated with subsequent wheezing. In contrast, it has been demonstrated that disease severity has a significant correlation with viral load or age in children with RSV infections [18]. In children with RSV infections, viral load and age may be associated with disease severity at the time of admission, but subsequent wheezing may be associated not only with disease severity at the time of admission but also other factors, such as the atopic predisposition. It has been reported that RV-infected children have a greater number of blood eosinophils and a higher prevalence of atopy than RSV-infected children [22, 25]. In the current study, it was not determined why there were differences in the relationship between atopic predisposition and subsequent wheezing in children with RV and RSV infections, but it is possible that patients with atopic predisposition had severe disease, and severe viral infections induced allergic responses in the airways.

This study had limitations. Although a large group of patients was studied, the individual number of patients was low, especially when the individuals were further subdivided into wheezing and no wheezing or subsequent wheezing and no subsequent wheezing cohorts. The reliability of the RV-detection rate may be questioned, because RV is frequently detected in symptom-free individuals [44].

In conclusion, the current study detected viruses from patients with wheezing who were hospitalized for lower respiratory tract infections; assessed the rate of wheezing, age, and severity of viral infections; and determined the presence of wheezing after discharge. It is hoped that similar research will be promoted to help understand the mechanism underlying the development of wheezing after a viral infection; the

findings of which may be useful for early intervention with asthma therapy and improvement in prognosis.

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