

# Factors Contributing to Symptom Duration and Viral Reduction in Outpatient Children With Respiratory Syncytial Virus Infection

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**Background:** We investigated the association between age, duration of clinical symptoms and viral shedding in outpatient children infected with respiratory syncytial virus (RSV) in Japan.

**Methods:** Outpatients younger than 2 years of age, with suspected RSV infection between 2014 and 2018, were enrolled in the study. Following informed consent, nasal samples were collected at first and second clinic visits (with 0–9 days gap). RSV-A or -B infection and viral load were determined by real-time polymerase chain reaction. Clinical symptoms were recorded at first clinic visit, and fever and symptoms were recorded at home for up to 8 days. Association between clinical symptoms and patient characteristics, such as age, sex and birth weight, were analyzed using ordered logistic regression analysis. The association between viral reduction and estimated shedding period was examined using linear regression analysis.

**Results:** Among the 205 cases enrolled in the study, no difference was found in patient characteristics between RSV-A and -B infection. Duration of fever was prolonged with increased age. Duration of rhinorrhea and cough was shorter in females than in males and in groups with birth weight  $\geq 3$  kg than in those with  $< 2.5$  kg. Daily viral reduction increased and estimated viral elimination period decreased with age.

**Conclusions:** Fever duration was found to increase while viral shedding decreased with patient age.

**Key Words:** respiratory syncytial virus, clinical symptoms, outpatient, viral reduction, children

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Respiratory syncytial virus (RSV) is abundant in winter and may cause bronchiolitis or pneumonia in children.<sup>1</sup> Most children are infected at or before 2 years of age.<sup>2</sup> Infants that are preterm or have congenital heart diseases or chronic lung diseases are at a higher risk of lower respiratory tract infection and hospital admission.<sup>3–5</sup> The risk factors associated with acute lower respiratory tract infection due to RSV include preterm birth, low-birth weight, male gender, sibling number, maternal smoking, history of atopic dermatitis, non-breast-feeding and crowding.<sup>6</sup> Among the serotypes A and B of RSV, the clinically more harmful one remains controversial.<sup>7</sup>

Currently, palivizumab is approved for use in preterm infants and is useful in decreasing ICU or pediatric ward admission.<sup>8,9</sup> As neither an antiviral medicine nor a vaccine for RSV infection is available, a better understanding of the clinical course of RSV infection and viral dynamics in vivo is considered necessary.

However, research regarding the clinical course of RSV infection in outpatients or viral reduction of RSV is limited. Although the association between primary symptoms and patient background information had been previously reported,<sup>10</sup> symptom duration in outpatients infected with RSV remains unclear. Therefore, we aimed to identify the patient characteristics that contribute to clinical RSV symptoms and viral reduction in RSV-infected children.

## MATERIALS AND METHODS

### Study Design and Participants

We conducted a prospective observational study on children with suspected RSV infection at 12 clinics and hospitals across Japan: Hiraoka Kohen Children's Clinic in Hokkaido, Tomimoto Children's Clinic in Aomori, Kaji Clinic in Tokyo, Sano Children's Clinic in Niigata, Shizuoka Kousei Hospital in Shizuoka, National Mie Hospital in Mie, Saito Children's Clinic in Shiga, Aoki Children's Clinic in Nara, Suzuki Children's Clinic in Yamaguchi, Nagai Children's Clinic in Kagawa, Shimada Children's Clinic in Kumamoto and Awase Daiichi Clinic in Okinawa.

Patients visited the clinic or hospital between August and February every year from 2014 to 2018. Children underwent RSV rapid diagnostic test (RDT) if they were under 6 years of age and were clinically suspected of RSV infection. Children were enrolled in the study irrespective of whether they had a positive RDT if their parents provided informed consent. All collaborating clinics and hospitals were requested to enroll at least one RDT positive and one negative patient every week because of the funding restraint.

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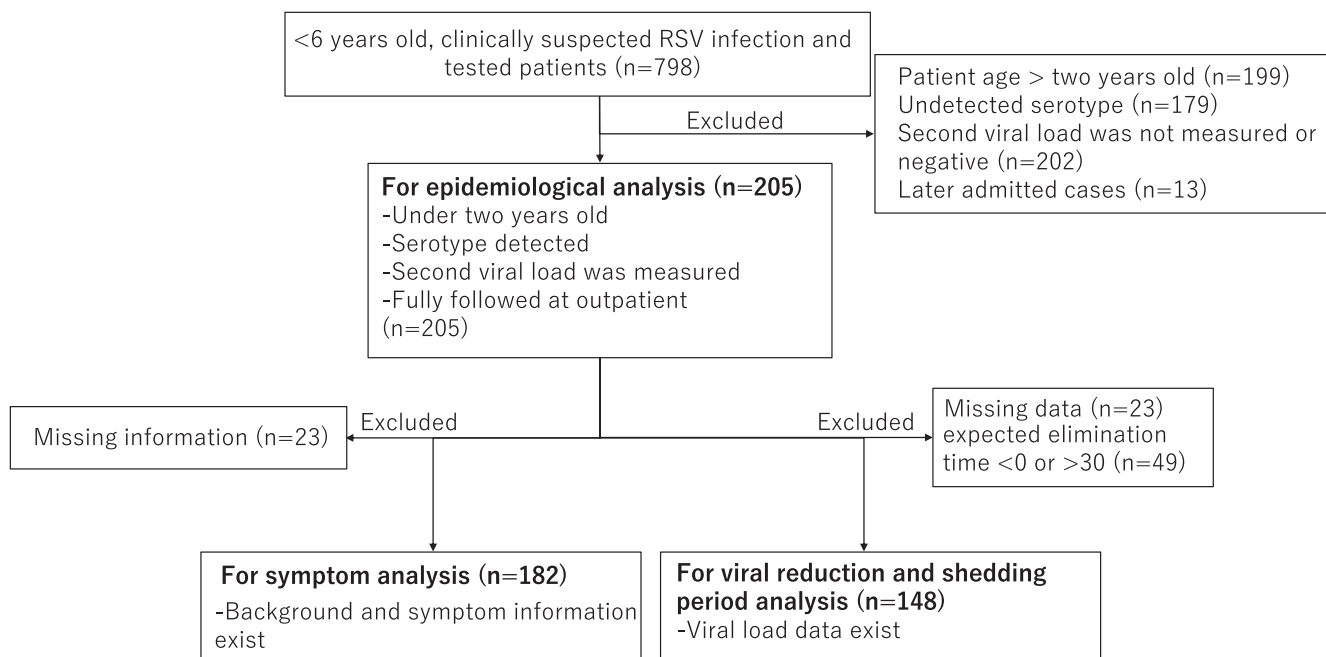
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**FIGURE 1.** Study design and inclusion criteria.

Of the children enrolled, selected children as per criteria shown in Figure 1 were analyzed. There was no admitted child included in the study.

### Clinical Records

Physicians were asked to record patient characteristics on registration sheets. Patient characteristics included age, sex, symptom onset date, first visit date, signs and symptoms, complications, background information (nursery use, household smoking and presence of siblings), birth information (gestational week and birth weight), history of RSV infection, history of palivizumab use and underlying diseases. Parents were asked to record the signs and symptoms of children for up to 8 days after the first visit. Information sheets were requested to return on the second visit or later. Parents checked patient body temperature three times a day. If body temperature measured  $\geq 37.5^{\circ}\text{C}$ , it was recorded as fever.

### Specimen Collection

Among enrolled children, nasal swabs were collected from patients regardless of RDT results. Similarly, specimens were collected at the second visit approximately after 0–9 days of the first visit.

RDTs for RSV used in this study were QuickNavi (DenkaSeiken, Tokyo, Japan), QuickNaviRSV (DenkaSeiken, Tokyo, Japan), PrimeCheckRSV (Alfresaphama, Osaka, Japan), Rapid-TestaRSV-Adeno (SekisuiMedical, Tokyo, Japan), ImunoAceRSV (Towns, Shizuoka, Japan) and CheckRSV (MeijiSeikapharma, Tokyo, Japan). Japanese public health insurance covered the use of all RDTs for children under one year of age in outpatient clinics. In our study, RDTs for patients older than 1 year were donated by Denka Seiken Co., Ltd.

Specimens collected were stored in the viral transport medium at  $-20^{\circ}\text{C}$  at each clinic or hospital. Samples were transported to Niigata University regularly within 1 month after collection. These specimens were then stored at  $-80^{\circ}\text{C}$  until further laboratory analysis.

### Viral Detection

For RSV subgroup detection and viral load measurement, RNA was extracted from the clinical specimens using the Extragen II kit (TOSOH, Tokyo, Japan). Complementary DNA (cDNA) synthesis was performed using random primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen Corp., Carlsbad, CA) incubated at  $37^{\circ}\text{C}$  for 1 hour. We identified RSV serotype A or B from cDNA using TaqMan probes targeting the nucleic acid sequences unique to serotype A or B in F protein of human RSV.<sup>11</sup> The primer and probe set specific for either RSV-A or RSV-B were included in the real-time polymerase chain reaction (PCR) run using appropriate reaction mixtures and cDNA to identify RSV-A or RSV-B. Amplification was conducted as follows:  $95^{\circ}\text{C}$  for 10 seconds, followed by 50 cycles of  $95^{\circ}\text{C}$  for 5 seconds and  $60^{\circ}\text{C}$  for 30 seconds. Positive Ct value threshold was 35 cycles while those  $>35$  cycles were considered negative for RSV-A and -B. To quantify viral load in a clinical sample, another real-time PCR assay was performed using the primer and probe set targeting a standard sequence for RSV-A and -B in M gene of RSV.<sup>11</sup> Real-time PCR was run in 25  $\mu\text{L}$  reaction mixtures using 12.5  $\mu\text{L}$  2  $\times$  Premix Taq solution, 1.25 U of TaKaRa Taq DNA Polymerase, 0.25  $\mu\text{L}$  each of forward and reverse primers (20 pmol/mL each), 1  $\mu\text{L}$  of 5 pmol/mL TaqMan probe, 1  $\mu\text{L}$  of cDNA template and 10  $\mu\text{L}$  nuclease-free water. The target sequence was cloned into a pM20-T vector (Takara Bio Inc., Shiga, Japan) using a Mighty TA cloning kit (Takara Bio Inc., Shiga, Japan) according to manufacturer's protocol to obtain positive controls. The cloned plasmid was quantified using a spectrophotometer and serially diluted. A standard curve was prepared for quantification using the simultaneous amplification of plasmid controls, 10 times serially diluted, ranging over 8 different concentrations from 3.19 copies/ $\mu\text{L}$  to  $3.19 \times 10^7$  copies/ $\mu\text{L}$ . Each sample and quantification control were run in duplicate. Viral load of each sample was calculated from the standard curve. VLs less than 10 copies/ $\mu\text{L}$  were regarded as negative. All reactions were performed using a Dice Real-Time PCR Systems TP800 thermocycler (Takara Bio Inc., Shiga, Japan).

### Calculation of VL

Daily viral reduction and viral shedding period were calculated as previously reported<sup>12</sup>:

$$\text{Daily viral reduction} = \ln(V_0 / V_t) \times \frac{1}{t}$$

where  $V_0$ ,  $V_t$  and  $t$  denote the first viral load, second viral load, and the interval between the first and second loads, respectively.

### Estimation of Viral Shedding Time

We defined the viral shedding period as the time from the first viral load measurement until the viral loads were below detection levels by RT-PCR (approximately 10 copies/reaction). We set  $V_t = 10$  copies/reaction and  $t =$  daily viral reduction ( $CL_v$ ) in the equation above to calculate the viral shedding period:

$$\text{Viral shedding period} = \ln(V_0 / 10) \times \frac{1}{CL_v}$$

where  $V_0$  denotes initial viral load.

### Definition of Variables

Patient characteristics were categorized as follows: age (<6, 6–12, 12–18 and 18–24 months); sex (male and female); gestational weeks (<37, 37–39 and  $\geq 39$ ); birth weight (<2500, 2500–3000 and  $\geq 3000$  grams); nursery use (yes or no); household smoking (yes or no); siblings (yes or no) and history of hospital admission (yes or no). Durations of clinical symptoms were categorized and coded as follows: fever (none as 0, 1–2 days as 1 and  $\geq 3$  days as 2); cough and rhinorrhea duration (none as 0, 1–5 days as 1 and  $\geq 6$  days as 2); duration of wheezing (none as 0, 1–3 days as 1 and  $\geq 4$  days as 2); duration of retraction and ill feeling (none as 0,  $\geq 1$  day as 1) and duration of appetite loss (none as 0, 1–4 days as 1 and  $\geq 5$  days as 2).

### Statistical Analysis

The  $\chi^2$  tests were used to compare characteristics between patients infected with serotypes A and B. Bar graph was drawn to show the distribution of RSV serotype over four seasons. Ordered logistic regression analysis was performed to investigate the association between background characteristics and symptom duration. Linear regression analysis was used to analyze the relationship between characteristics and both viral reduction and viral shedding periods. A box plot of viral load reduction in each age group was drawn. Kruskal-Wallis analysis was performed for the comparison of daily viral reduction in each age group. The univariate analysis was used to investigate the association between the first viral loads and each symptom duration. All statistical tests were performed using Stata 15 (StataCorp LLC, College Station, TX).

### Ethics Statement

This study was approved by the ethics committee of Niigata University on 27 October 2014 (Acceptance number 2020).

## RESULTS

A total of 798 patients were enrolled in our study, and 205 cases were analyzed (Fig. 1). Of these, 122 were diagnosed as serotype A and 83 as serotype B. The distribution of serotypes in each year from 2014 to 2018 is shown in Figure 2. Serotype A was predominant in most years while serotype B was predominant in 2016. The background information of patients between serotypes showed that nursery use was significantly higher in case of serotype B compared with that in serotype A (52% vs. 34%,  $P = 0.02$ ) (Table 1). No other significant difference was seen between the serotypes.

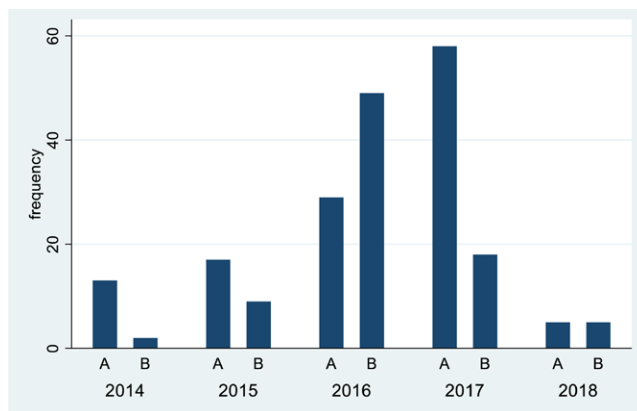


FIGURE 2. Distribution of respiratory syncytial virus serotype A and serotype B by the year.

### Clinical Course and Patient Characteristics

Of 205 cases, 182 were analyzed by ordered logistic regression analysis to see the association between clinical course and serotype or patient characteristics (Fig. 1, Table 2 and Table, Supplemental Digital Content 1, <http://links.lww.com/INF/D797>). Patients with RSV serotype B showed a shorter duration of appetite loss than those with RSV serotype A, but the rest of the symptoms did not have a difference between the 2 serotypes. Fever duration was longer in patients 6 to 12 months old and 12 to 18 months old than in those less than 6-months-old, respectively [odds ratio = 2.94, 95% confidence interval (CI) = 1.30–6.63 and odds ratio = 4.29, 95% CI = 1.68–10.93]. Fever duration was shorter in females than in males (odds ratio = 0.51, 95% CI = 0.28–0.93) and longer

TABLE 1. Comparison of Background Information in Serotype Groups

	Serotype A (n = 122)	Serotype B (n = 83)	P
Age (n = 205)			0.14
<6 months	38/122 (31.1)	14/83 (16.9)	
6 to <12 months	36/122 (29.5)	28/83 (33.7)	
12 to <18 months	31/122 (25.4)	27/83 (32.5)	
18 to <24 months	17/122 (13.9)	14/83 (16.9)	
Gestational weeks (n = 194)			0.22
<37 weeks	5/115 (4.3)	8/79 (10.1)	
37 to <39 weeks	40/115 (34.8)	22/79 (27.8)	
$\geq 39$ weeks	70/115 (60.9)	49/79 (62.0)	
Birth weight (n = 195)			0.62
<2500 grams	9/116 (7.8)	8/79 (10.1)	
2500 to 3000 grams	42/116 (36.2)	32/79 (40.5)	
$\geq 3000$ grams	65/116 (56.0)	39/79 (49.4)	
Daycare use (n = 189)			0.02
No	74/112 (66.1)	37/77 (48.1)	
Yes	38/112 (33.9)	40/77 (51.9)	
Sex (n = 200)			0.39
Male	71/119 (59.7)	43/81 (53.1)	
Female	48/119 (40.3)	38/81 (46.9)	
Sibling (n = 197)			0.09
No	36/118 (30.5)	34/79 (43.0)	
Yes	82/118 (69.5)	45/79 (57.0)	
Household smoking (n = 199)			1.00
No	62/118 (52.5)	43/81 (53.1)	
Yes	56/118 (47.5)	38/81 (46.9)	

Number of cases with missing data: 11 for gestational weeks, 10 for birth weight, 16 for daycare, 5 for sex, 8 for sibling and 6 for household smoking.

in patients with household smoking (odds ratio = 2.58, 95% CI = 1.42–4.71).

For other characteristics analyzed; females showed shorter period of coughing and rhinorrhea than males, the groups at 37–39 gestational weeks showed shorter duration of wheezing than the groups at less than 37 gestational weeks, the group with a birth weight of more than 3000 grams showed shorter period of coughing and rhinorrhea than those less than 2500 grams, and the groups being nursed had a longer duration of wheezing than the ones that were not being nursed (Table 2 and Table, Supplemental Digital Content 1, <http://links.lww.com/INF/D797>).

**Viral Reduction and Viral Shedding Periods**

Of 205 cases, 148 were analyzed using univariate and multivariate linear regression (Figure 1 and Table 3). In univariate analysis, the viral reduction was not significantly different across age groups (Figure, Supplemental Digital Content 2, <http://links.lww.com/INF/D798>). However, in multivariate analysis, daily viral reduction increased concurrently with age (Table 3). In turn, the viral shedding period was decreased by roughly one day as the age of the group increased. There was no significant difference in viral reduction or shedding based on gender, gestational week, birth weight or serotype.

**VL at the First Visit and Symptom Duration**

There was no correlation between the VL at the first visit and the duration of symptoms, such as fever, cough, wheezing, rhinorrhea, retraction, appetite loss and ill-feeling (Figure, Supplemental Digital Content 3, <http://links.lww.com/INF/D796>).

**DISCUSSION**

In this study, we examined RSV-infected outpatients under 2 years of age in Japan over a period of 4 years, to investigate the association of patient characteristics and serotypes with the duration of various clinical symptoms or viral load reduction and period of shedding, along with the relationship between symptom duration in patients and the viral load at first visit.

There was no difference between RSV serotype (A or B) and patient characteristics, except for nursery use. Study on hospitalized patients reported serotype A infected children to be significantly younger than serotype B infected ones.<sup>13</sup> However, the clinical characteristics by serotype may be varied by the target population or genotypes of the strains that circulated in the year.

Our data, including only outpatient children, showing no association between patient characteristics and serotypes, suggested RSV serotype not to be significantly related to clinical

**TABLE 2.** Results of Ordered Logistic Regression Analysis for the Association Between Background Information and Duration of Symptoms in Respiratory Syncytial Virus Infection

	Fever	Cough	Wheeze	Rhinorrhea	Retraction	Appetite Loss
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Serotype B	0.82 (0.45–1.48)	0.79 (0.42–1.49)	0.80 (0.44–1.45)	1.01 (0.54–1.90)	0.67 (0.32–1.38)	0.51* (0.28–0.92)
Age group						
6 to <12 months	2.94* (1.30–6.63)	1.09 (0.47–2.53)	1.38 (0.64–2.98)	0.97 (0.41–2.25)	0.85 (0.35–2.08)	1.30 (0.58–2.89)
12 to <18 months	4.29* (1.68–10.93)	0.61 (0.23–1.58)	0.61 (0.26–1.43)	0.62 (0.24–1.63)	0.31* (0.10–0.95)	1.15 (0.47–2.84)
18 to <24 months	1.45 (0.54–3.92)	0.77 (0.27–2.17)	0.50 (0.18–1.37)	1.00 (0.35–2.84)	0.42 (0.12–1.39)	1.52 (0.56–4.12)
Female	0.51* (0.28–0.93)	0.32* (0.17–0.61)	0.67 (0.38–1.19)	0.34* (0.18–0.64)	0.97 (0.48–1.94)	0.63 (0.35–1.14)
Gestational week group						
37 to <39 weeks	0.65 (0.17–2.50)	0.19 (0.03–1.14)	0.19* (0.04–0.88)	0.17 (0.03–1.05)	0.40 (0.08–1.86)	0.30 (0.07–1.28)
≥39 weeks	1.17 (0.29–4.67)	0.33 (0.05–2.05)	0.31 (0.06–1.51)	0.26 (0.04–1.60)	0.83 (0.17–4.02)	0.30 (0.07–1.32)
Birth weight (g)						
2500 to <3000	0.75 (0.22–2.57)	0.31 (0.07–1.43)	0.56 (0.15–2.12)	0.25 (0.05–1.18)	0.45 (0.12–1.74)	0.49 (0.13–1.79)
≥3000	0.64 (0.17–2.36)	0.20* (0.04–0.99)	0.41 (0.10–1.63)	0.20* (0.04–0.97)	0.33 (0.08–1.41)	0.67 (0.17–2.57)
Nursery	1.90 (0.99–3.66)	1.95 (0.97–3.92)	1.94* (1.02–3.70)	1.39 (0.71–2.75)	1.64 (0.74–3.62)	1.08 (0.57–2.05)
Smoking	2.58* (1.42–4.71)	1.26 (0.68–2.34)	1.70 (0.96–3.01)	1.07 (0.58–1.98)	1.07 (0.54–2.13)	0.89 (0.50–1.59)
Sibling	0.83 (0.44–1.57)	0.83 (0.43–1.62)	0.91 (0.49–1.69)	0.73 (0.37–1.41)	0.97 (0.46–2.04)	0.80 (0.43–1.47)

References are serotype A in serotype, <6 months in age group, male in sex, <37 weeks in gestational week group, <2500g in birth weight group, not using nursery in nursery, not smoking in smoking, not having sibling in sibling.  
\*P < 0.05.

**TABLE 3.** Results of Multiple Regression Analysis for Viral Reduction and Viral Shedding Periods

	Viral Reduction (Log Copy Number/Day)		Viral Shedding Periods (Day)	
	Coefficient (95% CI)	P	Coefficient (95% CI)	P
Age group	0.10* (0.01 to 0.19)	0.02	-0.96* (-1.86 to -0.06)	0.04
Gestational week group	-0.05 (-0.22 to 0.12)	0.57	0.82 (-0.91 to 2.55)	0.35
Birth weight group	0.16 (-0.01 to 0.329)	0.06	-1.64 (-3.30 to 0.01)	0.05
Serotype				
Serotype B	0.11 (-0.07 to 0.29)	0.22	-0.57 (-2.38 to 1.25)	0.54
Sex				
Female	0.01 (-0.17 to 0.19)	0.91	0.30 (-1.51 to 2.11)	0.74

Age group was defined as <6, 6–12, 12–18 and 18–24 months and scored as 1, 2, 3 and 4, respectively. Gestational week group was defined as <37, 37–39 and >39 weeks and scored as 1, 2 and 3, respectively. Birth weight group was defined as <2500, 2500–3000 and >3000 grams and scored as 1, 2 and 3, respectively. References were serotype A in serotype and male in sex.  
\*P < 0.05.



severity. While some reports have shown increased severity for serotype A,<sup>14</sup> others have reported higher severity for serotype B.<sup>15</sup> Additional reports have shown equal severity for both serotypes A and B.<sup>16</sup> Thus, the questions on severity by serotype remain controversial. Possible reasons for the inconsistency in studies include the difference in study design, patient population, and methods of sampling and analysis. Most studies had been performed in only inpatients<sup>13</sup> or in both inpatients and outpatients.<sup>17</sup>

Our results of longer fever duration with increased age are in line with previous studies. Previous reports had demonstrated fever duration to be longer with age and frequency of high fever to also increase concurrently with age.<sup>18</sup> Therefore, fever may not be a prominent symptom, especially when children less than 6-month-old are infected with RSV. Thus, RSV infection should be considered at diagnosis even if an infant has a short fever duration but with other symptoms such as wheezing or rhinorrhea that are typical for RSV infections.

We observed daily viral reduction to increase with age in RSV-infected outpatients significantly. Most of the studies on viral load dynamics were reported from inpatients because serial sampling is more convenient for inpatient than outpatient.<sup>13,17</sup> The previous study in hospitalized patients revealed viral reduction to be slower at a younger age. However, the findings were not statistically significant.<sup>19</sup> Another study on inpatients showed that the period of viral shedding was longer with those less than 1-year-old than ≥1-year-old for RSV serotype A infection.<sup>20</sup> Our results showed similar findings despite in outpatients. Although the reason why the duration of viral shedding was prolonged in younger patients was not apparent, it may be related to the maturation of host immune response by growth and the faster viral clearance at the time of the reinfection in older children. Therefore, RSV-infected children at a younger age, especially less than 1-year-old, should be controlled for infection more than others because those children have a higher chance to infect other people who take care of them.

Our data showed no association between the first viral load and each symptom duration upon RSV infection. Viral loads at enrolment were reported to be inversely correlated with the Clinical Disease Severity Score in outpatients in the previous study.<sup>17</sup> Clinical Disease Severity Score comprised of 5 parameters, including respiratory rate, auscultation, transcutaneous oxygen saturation, retractions and level of activity. The higher score (0–15) indicates the more severe condition.<sup>21</sup> The previous study suggested that children with higher viral load at the first clinic visit showed less-severe clinical manifestation in outpatients. It could be possible that higher RSV loads promote an early robust innate immune response that can protect from disease progression.<sup>17</sup> The first viral load may be the determinant of initial severity of illness, although our study indicated that it might not be associated with the duration of symptoms.

Our results demonstrated a prolonged fever duration in patients with household smoking. Maternal smoking is a well-known risk factor for acute lower respiratory RSV infection.<sup>6</sup> Household smoking is also associated with otitis media in children.<sup>22</sup> Though otitis media is a known RSV complication, we did not find a significant association between prolonged fever and otitis media. Importantly, both household smoking and RSV infection increase the risk of otitis media. Thus, these factors may cause prolonged fever duration. Parents of infected children should be educated regarding the increased risks and prolonged fever of RSV infection due to smoking.

Our finding of fever, cough and rhinorrhea duration being shorter in females than in males is consistent with previous reports. Gender is an RSV admission risk factor.<sup>23</sup> Being male was also significantly associated with RSV-associated acute lower respiratory infection.<sup>6</sup> Thus, female patients may have a lower risk of admission

and less-severe RSV. Thus, RSV infection in male patients should be followed more carefully.

Our results regarding shorter wheezing duration at later gestational weeks also agree with the previous reports. The bronchiolitis severity score, including wheezing and retraction, was significantly higher in preterm infants than in term infants, in studies of children with RSV bronchiolitis.<sup>24</sup> Adjusted forced expiratory volume in a second was lower in the preterm born group than in the term born group even in the adolescent period.<sup>25</sup> Our data, therefore, suggested gestational period to influence the disease outcome most probably. Similarly, we observed a shorter cough and rhinorrhea duration with higher birth weight, which corroborates with previous reports. The odds ratio of acute lower respiratory tract RSV infection was 1.9 in low-birth weight infants (LBWI) than in non-LBWI in a previous report.<sup>6</sup> This gives strong evidence for LBWI to be administered of palivizumab.

Our study had some limitations. First, the procedure of collecting nasal specimen was not standardized and might affect the viral load measurement. Second, parents were asked to record patient symptoms; although it is easy to assess fever, cough and rhinorrhea, it might be difficult for them to determine retraction. Additionally, appetite loss and “ill feeling” are subjective symptoms compared with cough or rhinorrhea, and hence, the duration of these symptoms may not be more objective. Finally, the viral reduction was calculated using 2 viral load measurements because the serial sampling was difficult for outpatients. Calculations using more than two measurements might be more accurate.

Despite the limitations, our study revealed that several patient characteristics were related to the duration of symptoms in RSV-infected infants. Therefore, we suggest that age, household smoking, gender, gestational age and birth weight should be considered in the diagnosis and treatment of RSV infection. Gestational age is an essential factor in applying palivizumab to preterm infants. Our study suggested that viral loads are higher in younger children. This is important when we consider infection control measurements for RSV-infected patients. Our results will help healthcare professionals to diagnose and manage RSV infection appropriately in young children.

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