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# Phenotypical Characterization of Human Rhinovirus Infections in Severely Premature Children

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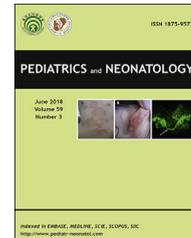
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Original Article

# Phenotypical characterization of human rhinovirus infections in severely premature children



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## Key Words

human rhinovirus;  
hypoxemia;  
prematurity;  
wheezing

**Background:** Human Rhinovirus (HRV) has been identified as the most common cause of acute respiratory infections and hospitalizations in premature children. It is unclear if premature children are more susceptible to HRV due to their decreased pulmonary reserve or because they have enhanced lower airway reactivity to HRV.

**Methods:** We conducted a retrospective analysis of the clinical respiratory presentation of all PCR-confirmed HRV infections in full-term and premature children aged  $\leq 3$  years in our institution. Standardized respiratory distress scores were developed to examine lower airway obstruction (i.e., wheezing, hyperinflation, and sub-costal retractions) along with markers of decreased pulmonary reserve (hypoxemia and tachypnea) in young children with HRV infections. Demographic and clinical variables were obtained from reviewing electronic medical records (EMR).

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**Results:** This study included a total of 205 children; 71% of these children were born full-term (>37 weeks gestation), 10% preterm (32–37 weeks) and 19% severely premature (<32 weeks). Our results demonstrated that: 1) HRV infections in the first 3 years of life were associated with higher overall respiratory distress scores in severely premature children relative to children born preterm or full-term; 2) HRV-infected severely premature children  $\leq 3$  years old were more likely to have lower airway obstruction than HRV-infected children born preterm or full-term; and 3) other clinical signs of respiratory distress such as tachypnea and hypoxemia were not more common in severely premature than in preterm and full-term children during an HRV infection.

**Conclusions:** Our results indicate that HRV infections in severely premature children are associated with lower airway obstruction rather than hypoxemia or tachypnea. The latter suggests that enhanced airway reactivity is the underlying mechanism for the increased susceptibility to HRV in severely premature children. Longitudinal studies are needed to understand why premature babies develop airway hyper-reactivity to HRV and the long-term effects of early HRV infection in this population.

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## 1. Introduction

Human rhinovirus (HRV) is the most common cause of asthma exacerbation in all ages<sup>1</sup> and recent evidence demonstrates that HRV causes severe respiratory infections in premature children.<sup>2–4</sup> In fact, HRV is the most common reason for hospitalization in this population.<sup>4</sup> Despite the clinical connection between HRV and prematurity, it is still unclear if premature children are more susceptible to severe HRV infections due to their underlying decreased pulmonary reserve or because premature birth itself increases the susceptibility to develop enhanced airway reactivity triggered by HRV.

Prematurity is associated with chronic lung disease (CLD) characterized by hypoxemia due to abnormal alveolarization leading to simplified gas-exchange units.<sup>5</sup> Interestingly, in addition to CLD, premature birth is increasingly recognized as a major risk factor for the development of the asthmatic condition,<sup>6,7</sup> although the underlying mechanisms of this association are presently unknown. It is also well-established that premature children are at high risk of developing severe lower respiratory tract infections caused by paramyxoviruses such as Respiratory Syncytial Virus (RSV) and Human Metapneumovirus (HMPV), which exacerbate underlying hypoxemia and CLD.<sup>8,9</sup> In contrast to RSV or HMPV, which have been shown to induce lung injury and increased airway oxidative stress,<sup>10</sup> HRV is mostly associated with the induction of Th2-driven allergic inflammation leading to airway hyper-reactivity (AHR) and asthma exacerbations.<sup>1</sup>

The aim of this paper is to describe the clinical phenotype of HRV infections in hospitalized young children and to better understand the potential mechanisms by which this virus causes severe respiratory disease in this vulnerable population of severely premature children. Given that the pathogenic role of HRV was primarily limited to the induction and/or exacerbation of AHR in high-risk groups (e.g., asthma, COPD),<sup>1</sup> our hypothesis was that in the group of young children requiring hospitalization due to HRV infection, those born severely premature (<32 weeks gestational age), would have a significantly higher probability to present signs of lower airway obstruction (e.g., wheezing) and

air trapping/hyperinflation (e.g., sub-costal retractions) relative to full-term individuals. In contrast, we postulated that other signs of respiratory distress not typically associated with AHR, including hypoxemia and tachypnea, would be similarly present in premature vs. full-term children hospitalized with HRV infection.

## 2. Materials and methods

### 2.1. Study subjects

We conducted a retrospective cross-sectional analysis of a cohort of children  $\leq 3$  years of age admitted with HRV infection, confirmed by PCR analysis, to the Children's National Medical Center (CNMC) in 2014. Viral PCR was performed on subjects who presented to the hospital with suspected viral respiratory tract infection at the discretion of the clinician. We included children with positive PCR for HRV and excluded individuals with mixed viral infections (HRV mixed with other viruses) to only focus on HRV. Patients with significant co-morbidities such as cardiorespiratory conditions (other than prematurity), genetic syndromes and immunosuppression were excluded from the study. This study was approved by the Institutional Review Board at the Children's National Medical Center.

### 2.2. Clinical and demographic variables

Clinical and demographic variables were obtained by reviewing electronic medical records (EMR) at CNMC. Demographic variables comprised gestational age in weeks, age, gender, and ethnicity. Other clinical variables included tachypnea, retractions, abnormal breath sounds (wheezing), oxyhemoglobin saturation values by pulse oximetry (SaO<sub>2</sub>), and supplemental oxygen (O<sub>2</sub>) requirement relative to patient's baseline. For the purpose of the study, clinical parameters were characterized as binary outcomes for the following: severe prematurity defined *a priori* by a gestational age of less than 32 weeks to include extremely preterm and very preterm subjects based on World Health

Organization (WHO) definition of prematurity<sup>11–13</sup> and oxygen supplementation. Tachypnea was stratified and scored in groups (0–3) according to respiratory rate definitions used in bronchiolitis scores as follows: 0 for <30 breaths per minute (bpm); 1 for 30–45 bpm; 2 for 46–60 bpm and 3 for >60.<sup>8,14,15</sup> Stratified wheezing severity (0–3) was also defined based on distress scores<sup>14</sup> as 0 for none; 1 for expiratory only; 2 for inspiratory ( $\pm$ expiratory) and 3 for audible without stethoscope or silent chest (minimal or no air entry) = 3.

### 2.3. Clinical evaluation of viral respiratory tract infection severity based on lower airway obstruction and respiratory distress

As previously described,<sup>8</sup> to retrospectively assess overall clinical severity of HRV infection, we recorded wheezing, retractions, supplemental O<sub>2</sub> needed and tachypnea at the initial presentation based on EMR and combined these into the respiratory distress score (0–10). For this score, we used a stratified value for tachypnea (0–3) and combined

assigned values with the binary need of O<sub>2</sub> (0–2), stratified presence of wheezing (0–3) and retractions (0–2), yielding a total maximal value of 10 points (Fig. 1). Of note, the four clinical variables included in this respiratory distress score were selected because they represented the main phenotypical features of viral bronchiolitis in children,<sup>8,16</sup> lower airway obstruction and respiratory distress, and they were the parameters included in bronchiolitis scores validated by our group (Modified Wood's Clinical Asthma score [M-WCAS] and Tal severity score).<sup>8,14,15</sup>

### 2.4. Statistical analysis

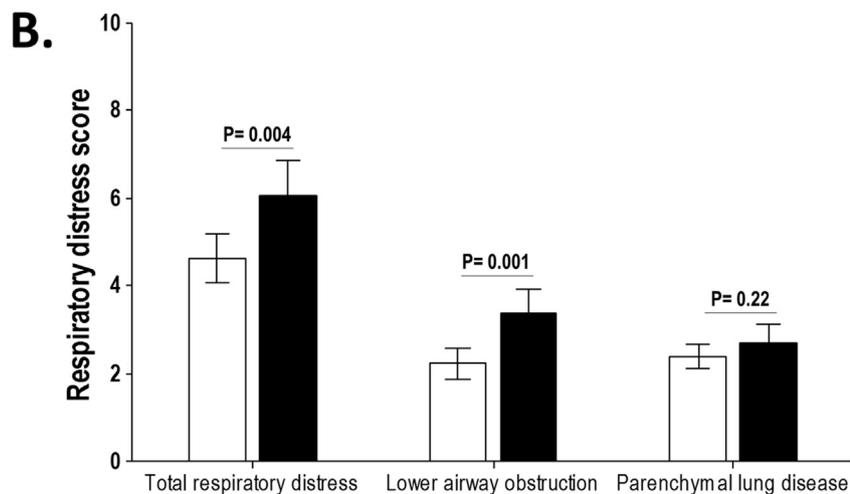
Data were analyzed using the software SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used to calculate the prevalence of HRV. Collected demographic and clinical data were compared with the use of a Chi-square test (categorical variables) or T-test or Wilcoxon rank-sum test as appropriate for continuous variables. Comparisons between GA groups were done with ANOVA and post-hoc Bonferroni adjustment. Significance was taken at the  $P < 0.05$  level.

## A. Respiratory Distress Score during Viral Infection

| Score | Subcostal Retractions | Wheezing | Respiratory Rate | Supplemental O <sub>2</sub> + |
|-------|-----------------------|----------|------------------|-------------------------------|
| 0     | None                  | None     | <30              | None                          |
| 1     |                       | (+)      | 30-45            |                               |
| 2     | (+)                   | (++)     | 46-60            | (+)                           |
| 3     |                       | (+++)    | >60              |                               |

Lower airway obstruction assessment

Parenchymal lung disease assessment



**Figure 1 Respiratory distress assessment during Rhinovirus infection.** A. parameters used to examine total respiratory distress and individual contribution in lower airway obstruction and parenchymal lung disease. Wheezing was stratified as (+) expiratory only; (++) inspiratory ( $\pm$ expiratory) and (+++) for audible without stethoscope or silent chest (minimal or no air entry); B. Respiratory distress assessment in children <3 yr of age hospitalized with rhinovirus infection (n = 205). Black bars represent subjects born <32 wks gestational age; white bars correspond to individuals born >32 wks. Data presented as 95% confidence interval of the mean.

### 3. Results

#### 3.1. Rhinovirus a common cause of hospitalization in premature children

We first examined the proportion of children born before term at 37 weeks gestation in our study group of hospitalized children with PCR-confirmed HRV infection. Among the 205 subjects included in this study, we identified that 34% (n = 69) of children hospitalized with HRV infection were born before term gestation. The majority of these premature children were born at less than 32 weeks gestation (22%; n = 47). We did not identify significant differences in the baseline characteristics, including demographics and family history of asthma, of the children born full term (>37 weeks gestation), preterm (32–37 weeks gestation) or severely premature (<32 weeks gestation) except for black ethnicity, which was more common in the severely premature group. Comparison of the baseline characteristics of these three groups is presented in Table 1.

#### 3.2. Respiratory distress severity evaluation in children hospitalized with human rhinovirus infection

We previously validated bronchiolitis scores in hospitalized children with viral respiratory infections.<sup>14</sup> Taking the physiological parameters included in those scores, we recently developed a respiratory distress scale to be used retrospectively in hospitalized children with respiratory viral infections.<sup>8</sup> Fig. 2 illustrates the parameters included in our respiratory distress score. It includes four parameters balanced to equally cover two main domains: parenchymal lung disease assessment (i.e., tachypnea and hypoxemia) and lower airway obstruction assessment (i.e., wheezing and sub-costal retractions). Hypoxemia and tachypnea are signs of involvement of parenchymal alveolar gas-exchange units during viral infection.<sup>8,16</sup> Viral-induced wheezing is indicative of narrowed lumen in the conductive airways and sub-costal retractions is a sign of air-trapping/hyperinflation causing flattening of the diaphragm.<sup>8,16</sup>

We first used these respiratory distress parameters to examine the overall clinical severity of HRV in hospitalized children. As expected, we identified that individuals born <32 weeks gestation had overall higher distress severity (total score 6.3; 95% CI 5.6–7) relative to children born either full term or preterm (4.6; 95% CI 4.1–5.1). Then we

conducted phenotypical characterization using individual parameters for parenchymal lung disease assessment and lower airway obstruction assessment. This analysis showed that the lower airway obstruction component (wheezing and sub-costal retractions) was significantly increased in severely premature children relative to individuals born preterm or full term (Fig. 1B). By contrast, we did not observe significant differences in the parenchymal lung disease component (hypoxemia and tachypnea) (Fig. 1B). To confirm these findings we performed an alternative analysis in which we examined individually the degree of tachypnea or the probability of needing supplemental oxygen (Fig. 2A) as well as the absolute values of respiratory rate and oxygen saturation (SaO<sub>2</sub>) at presentation to the hospital during HRV infection (Fig. 2B). This secondary analysis did not identify statistically significant differences in respiratory rate or oxygenation parameters in severely premature children versus those born preterm or full term.

#### 3.3. Dose effect of the degree of prematurity and probability of wheezing in children hospitalized with rhinovirus infection

Recent evidence demonstrates that the degree of prematurity has a dose effect in assessing the risk of developing asthma.<sup>7</sup> We have also found that there is relative dose response of prematurity in the secretion of airway Th2 cytokines during HRV infection.<sup>13</sup> Specifically, the group of children born <32 weeks gestation had the strongest Th2 response to HRV, with gradual decrease in individuals born at 32–37 weeks gestation and Th2 response was even lower in full term children with HRV.<sup>13</sup> To investigate whether there was also a dose effect of prematurity in the degree of lower airway obstruction during HRV infection, we examined the probability of developing wheezing or sub-costal retractions during HRV infection as a function of GA. The data showed the probability of developing wheezing and sub-costal retractions increased with lower gestational age (Fig. 3), with the highest risk seen in the cluster of children born at <32 weeks gestation.

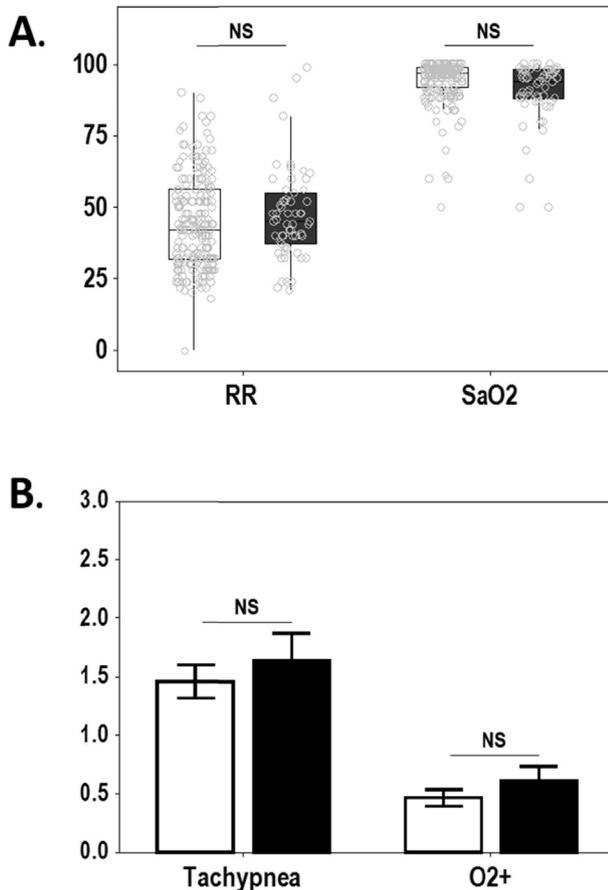
### 4. Discussion

Human rhinovirus infection (HRV) is the top cause of hospitalization in premature children.<sup>4</sup> It is unclear whether HRV causes severe respiratory disease in premature children primarily (due to the underlying parenchymal lung disease leading to worsening hypoxemia and tachypnea) or

**Table 1** Baseline characteristics for subjects.

|                                 | Term (>37 wks GA) | Preterm (32–37 wks GA) | Severe Premature (<32 wks GA) |
|---------------------------------|-------------------|------------------------|-------------------------------|
| N (%)                           | 135 (66)          | 22 (11)                | 47 (23)                       |
| Age (yrs.), mean (SD)           | 1.35 (1.0)        | 1.22 (0.8)             | 1.5 (1.0)                     |
| Male, n (%)                     | 78 (58)           | 14 (63)                | 33 (70)                       |
| Black, n (%)*                   | 64 (47)           | 6 (27)                 | 31 (66)                       |
| Family history of asthma, n (%) | 27 (20)           | 2 (9)                  | 6 (13)                        |

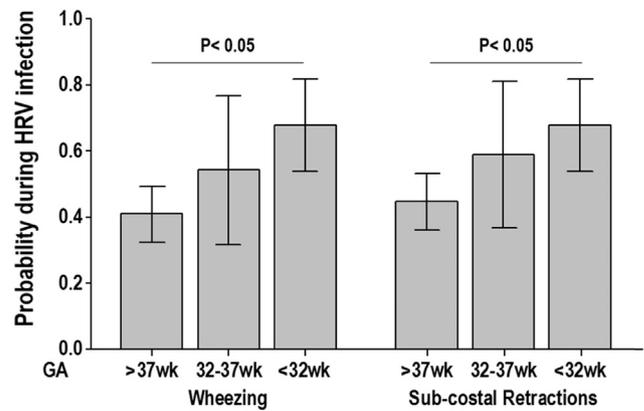
Demographics for all study subjects (n = 205) hospitalized with acute rhinovirus infection. GA = gestational age, SD = Standard deviation, \*P value < 0.05.



**Figure 2** Hypoxemia and tachypnea during Rhinovirus infection. A. Boxplots (median and interquartile ranges) of respiratory rate (RR) pulse-oximetry (SaO<sub>2</sub>) at admission during rhinovirus infection. B. Values of stratified tachypnea and need for supplemental O<sub>2</sub> during rhinovirus infection. Black bars represent subjects born <32 wks gestational age; white bars correspond to individuals born >32 wks. Data presented as 95% confidence interval of the mean. Data included all hospitalized children with rhinovirus infection (n = 205). NS = non-significant (p > 0.05).

whether HRV is mainly a trigger of wheezing and AHR in premature children similar to its well-established role in asthma exacerbations.<sup>1</sup> The main finding of our study is that premature children hospitalized due to HRV infection have primarily clinical signs of lower airway obstruction which are typically seen during viral-induced airway hyperactivity. This study provides new evidence that HRV infection in premature children, similarly to what has been previously described in the asthmatic condition,<sup>1</sup> seems to trigger AHR leading to wheezing, air trapping, and more severe respiratory disease which ultimately causes an increase in health care utilization<sup>2–4</sup> and increased risk for hospitalization.<sup>4</sup>

In susceptible individuals HRV is known to activate immune signaling pathways that promote Th2 airway inflammation<sup>17–21</sup> leading to increased mucus secretion and bronchoconstriction.<sup>17–21</sup> Animal models of allergic lung inflammation and human-based research have shown that HRV induces the secretion of airway epithelial Th2-



**Figure 3** Dose effect of prematurity in the probability of wheezing or sub-costal retractions during Rhinovirus infection. Data included all hospitalized children 0–3 years old with rhinovirus infection (n = 205). Data presented as 95% confidence interval of the mean by gestational (GA) clusters. P values obtained by ANOVA and post-hoc Bonferroni adjustment.

promoting cytokines such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33.<sup>17</sup> The secretion of these cytokines occurs after an innate recognition of receptors present in the airway epithelial cell including toll-like receptor (TLR)-3, MDA 5 and RIG-I,<sup>22</sup> which activate downstream interferon-responsive genes and other pro-inflammatory cytokines through the activation of NFKB signaling.<sup>22,23</sup> Interestingly, we have recently shown that premature infants have an airway immune response during viral infections that closely resembles the Th2-driven inflammation seen in the asthmatic condition.<sup>13,24</sup> As a result, premature infants have an increased airway secretion of the classical Th2 cytokines IL-4 and IL-13 in response to natural HRV infection.<sup>13</sup> Expanding these findings, our current study suggests that HRV induces AHR in premature infants, which is manifested as lower airway obstruction. Clinically, the best markers of AHR in infants are wheezing and hyperinflation<sup>16</sup> which are best assessed by auscultation during forced exhalation maneuvers and visual inspection of sub-costal retractions due to air-trapping leading to diaphragm flattening.<sup>16</sup> These clinical findings suggest that HRV effect in the lungs of premature infants is mostly due to mucosal inflammation and bronchoconstriction leading to lower airway obstruction, as recently suggested in animal models of early rhinovirus infection.<sup>17</sup>

Prior studies have investigated the presence of lower airway obstruction in premature children.<sup>25–28</sup> Premature birth is associated with alveolar simplification due to impaired secondary septation leading to reduced lung capacity as well as increased airway resistance due to altered alveolar airway radial traction.<sup>5</sup> These findings have been confirmed through the use of pulmonary function testing<sup>25–28</sup> and studies on the effects of viral infections<sup>28</sup> have shown airway resistance is significantly increased during respiratory infections in premature infants.<sup>28</sup> In addition, studies primarily based on the report of wheezing have identified HRV is associated with frequent health care utilization mostly due to recurrent lower airway respiratory symptoms.<sup>2</sup> To extend these prior investigations, we conducted the first phenotypical characterization of HRV infection in premature

infants requiring hospitalization, using standardized clinical scales for respiratory distress.<sup>8,14,15</sup> The respiratory distress scores used in our current study were based on widely used clinical scales to examine bronchiolitis severity which had been previously validated by our group in the setting of hospitalized children.<sup>14</sup> These clinical scales include different parameters such as hypoxemia – better quantified as need of oxygen relative to baseline<sup>8</sup> together with tachypnea (absolute and stratified).<sup>8,14,15</sup> It is important to emphasize that in clinical practice respiratory rate (and tachypnea) should be classified according the normal range of different age population. For research purposes we implemented a scoring system that could not take respiratory rate as a continuous variable; it needed to be coded in a numerical scale to allow combination with other clinical parameters (e.g., wheezing, retractions, O<sub>2</sub>). The reason why we selected this tachypnea stratification (<30, 30–60, >60 bpm = 0,1,2,3) was our prior studies demonstrated that alone and, in combination with other parameters, it had optimal performance in clinical scoring in young children of different age ranges.<sup>8,14,15</sup> Specifically, it has adequate criterion validity, adequate construct validity, adequate inter-rater agreement, adequate sensitivity to change, and appropriate usability for infants hospitalized for acute bronchiolitis.<sup>8,14,15</sup> Using this approach we have identified hypoxemia and tachypnea are strong predictors of the need for advanced support including high-flow devices, positive airway pressure, and mechanical ventilation in lower respiratory infections.<sup>8</sup> Our results showed that hospitalized premature infants with HRV had overall higher respiratory distress scores relative to full-term children hospitalized due to HRV infection. However, the increase in respiratory distress was not due to higher levels of hypoxemia or tachypnea, which would be indicative of decreased respiratory reserve, CLD or parenchymal lung disease leading to ventilation/perfusion (V/Q) mismatch. By contrast, we observed a clear difference in the markers of lower airway obstruction, wheezing and sub-costal retractions in premature children with HRV. Collectively, these findings indicated that the main difference in the presentation of HRV infection between premature and full-term hospitalized children was the presence of lower airway obstruction and not worsening of respiratory reserve with signs of parenchymal lung disease.

In the current study we categorized tachypnea/hypoxemia relating to parenchymal disease and wheezing/sub-costal retractions to lower airway obstruction (Fig. 1). It is important to clarify that there is no solid clinical evidence that tachypnea and hypoxemia relate to parenchymal disease and not to airway obstruction. Our scoring classification should not be extrapolated to all respiratory conditions and this categorization should be understood in the specific context of viral respiratory infections of infants. Our rationale was partly based on our recent HMPV clinical study in premature children,<sup>8</sup> in which we found that parenchymal disease (verified by CXR infiltrates) was associated with hypoxemia/tachypnea and not wheezing (clinical marker of lower airway obstruction). Hypoxemia/tachypnea predicted poor clinical outcome as it was linked to a greater probability of respiratory failure and the need for more advanced support in the intensive care unit.<sup>8</sup> We speculate that hypoxemia results from V/Q mismatching

and tachypnea from the reduction in lung compliance due to a localized parenchymal disease (infiltrate) during HMPV infection. In our current RV study we observed that wheezing predominated over hypoxemia/tachypnea in the same population (premature infants). These findings suggest that the respiratory distress assessment of viral infections of infants needs to consider hypoxemia/tachypnea and wheezing (e.g., lower airway obstruction) separately to delineate phenotypical differences that may have significant impact in clinical outcomes.

Another important finding of our current study was the presence of a robust dose–response association between degree of prematurity and the probability of wheezing in premature children hospitalized with HRV. As shown in Fig. 3, there was a direct relationship (dose-effect) between prematurity, expressed as gestational age, and the likelihood of wheezing HRV infections. The greatest probability of wheezing was identified in the group of “severe prematurity” with less than 32 weeks gestation. These data resemble the recently identified dose effect of prematurity and risk of asthma compared with term children, at ages 0–5 and 6–9 years.<sup>7</sup> This study included 2540 longitudinal cases from the Boston Birth Cohort, in which the investigators identified that there was a significant increase in the risk of developing asthma when prematurity was clustered according to gestational age (to refine the assessment of the degree of prematurity) with the highest odds of asthma being observed in premature children <32 weeks gestation (OR 6.2 [95% CI: 3.3–11.6]; P:0.001).<sup>7</sup> This is the same prematurity cluster we identified with the highest probability of wheezing during HRV infection. Given that HRV-induced wheezing in early life is the strongest risk factor for persistence of wheezing and subsequent development of asthma,<sup>29</sup> future studies are needed to investigate the interplay and potential additive effects of prematurity and HRV in the pathogenesis of the asthmatic condition. These studies should adjust for additional factors that may favor prematurity and severity of rhinovirus infections including differences in family economic status, sibling numbers and nutritional status. Future research should also include more objective functional parameters and biomarkers to better define respiratory phenotypes in premature infants at baseline and during acute respiratory infections.

In summary, our study provides new clinical evidence demonstrating HRV infections in severely premature children lead to lower airway obstruction as demonstrated by wheezing and hyperinflation rather than hypoxemia or tachypnea. The latter suggests enhanced airway reactivity is the underlying mechanism for the increased susceptibility to HRV in severely premature children. This novel finding adds to the increasing evidence that premature birth is associated with the pathogenesis of the asthmatic condition. Longitudinal studies are needed to understand why premature babies develop airway hyper-reactivity to HRV and what long-term effects of early HRV infection are in this population.

## Conflict of interest

The authors have no conflicts of interest relevant to this article.

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