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ORIGINAL ARTICLE: RESPIRATORY INFECTIONS

WILEY PEDIATRIC PULMONOLOGY

Interleukin-13 associates with life-threatening rhinovirus infections in infants and young children

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Abstract

Objective: Delineate risk factors associated with severe hypoxemia (O_2 sat ≤87%) in infants and children younger than 2 years hospitalized with single pathogen HRV infection.

Study Design: Prospective study in a yearly catchment population of 56 560 children <2 years old between 2011 and 2013 in Argentina. All children with respiratory signs and O_2 sat <93% on admission were included. HRV infections were identified by reverse transcriptase-polymerase chain reaction. Epidemiologic, clinical, viral, and immunological risk factors were assessed.

Results: Among 5012 hospitalized patients, HRV was detected as a single pathogen in 347 (6.92%) subjects. Thirty-two (9.2%) had life-threatening disease. Traditional risk factors for severe bronchiolitis did not affect severity of illness. HRV viral load, HRV groups, and type II and III interferons did not associate with severe hypoxemia. Interleukin-13 Levels in respiratory secretions at the time of admission (OR = 7.43 (3-18.4); $P < 0.001$ for IL-13 >10 pg/mL) predisposed to life-threatening disease.

Conclusions: Targeted interventions against IL-13 should be evaluated to decrease severity of HRV illness in infancy and early childhood.

KEYWORDS
biomarkers, viral, epidemiology

1 | INTRODUCTION

Human rhinovirus (HRV) infections are an important cause of hospitalization in infants and young children worldwide.[1] Although initially recognized as agents of the common cold,[2] HRV are now recognized as important mediators of pediatric and adult asthma exacerbations[3,4] and in association with asthma inception.[5] Recent studies have also shown HRV to be important agents of severe acute lung disease in premature babies,[6] term infants and young children.[7]

While risk factors for HRV-mediated asthma exacerbations and co-factors potentially associated with asthma inception have been extensively studied,[4,6] less is known about demographic variables that

Abbreviations: ARD, acute respiratory disease; HRV, human rhinovirus; IL-13, interleukin 13.

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modulate the risk of severe acute respiratory illness due to HRV in infants.[7] Previous studies identified maternal atopy and asthma in association with hospitalizations,[7] but whether a threshold in HRV respiratory load or certain immune profiles during acute illness contribute to severity is unclear. High IL-13 Levels have been associated with HRV-mediated wheezing in older patients.[8] Earlier work from others and our group revealed differences in the IFNλ_1 response to HRV infection in asthmatics versus healthy adults[9] and in children with varying severity of asthma exacerbations.[10] Asthmatic adults infected with HRV exhibited lower levels of IFNλ_1 associated with wheezing when compared to non-wheezing healthy controls in Britain.[9] In Argentina, levels of IFNλ_1 were dose-dependently higher in asthmatic children with increasing severity of exacerbations due to HRV infection.[10] However, the role of IL-13 and IFNλ_1 in life-threatening acute respiratory infections (ARI) in infants and young children is unknown.

Moreover, while routine HRV infections typically range from asymptomatic to self-limited hospital admissions of less severity than those elicited by other viral pathogens, a subgroup of hospitalized infants, and young children infected with HRV experience life-threatening disease. Importantly, epidemiological and biological variables predisposing to extreme, life-threatening illness are unknown. To delineate epidemiological, clinical, viral, and immune risk factors associated with life-threatening acute HRV infections in early life, we prospectively studied 347 HRV-infected infants and children hospitalized with ARI with hypoxemia between 2011 and 2013 in an yearly catchment population of 56 560 children younger than age 2 years in Buenos Aires, Argentina.

2 | METHODS

2.1 | Population and study period

We investigated a population we had studied previously[11] in a low socio-economic area of Buenos Aires, Argentina where there is an estimated population of 361 000.[12] In this area, the southern region has the lowest socioeconomic indicators and is home to 64 600 children younger than age 2.[12,13] There, we conducted a prospective study between 2011 and 2013 in a catchment population of ~56 560 children younger than 2-years old during the respiratory seasons (extending between detection by the hospitals of two positive cases of RSV-ARI in one of the 12 institutions in a week, until no RSV positive cases were admitted to four institutions in 1 week).[12,13] All these children lacked private medical insurance and received care from 12 public hospitals in Buenos Aires.[13] The study was approved by the institutional review boards at each participating institution. Informed consent was obtained from all participating parents or guardians.

Eligible patients were younger than 2 years of age. These children were admitted with a diagnosis of severe ARI, defined as the sudden onset of cough, wheezing, retractions and/or crackles with-or-without fever, and an oxygen saturation (O_2sat) <93% at rest when breathing room air.[14] All enrolled children were monitored daily during hospitalization using specifically designed forms until discharge.[15]

2.2 | Epidemiological and clinical data

Upon admission to the hospital and through their hospitalization, we collected epidemiological and clinical data using specifically designed questionnaires. Risk factors for hospitalization included birth-weight, lack of breastfeeding, sex, age, prematurity (<37 weeks gestation at birth), asthmatic parents, atopy in siblings, and precarious home.[15] Precarious home was defined as having a house with at least one of the following deficits: construction of tin/mud, dirt floor, low household income (monthly income < U$200) or lack of sewage. In addition, crowding (>3 persons/room), maternal educational level, malnutrition, and smoking at home were examined. Clinical variables included underlying chronic illness (pulmonary, cardiac, or neurological), intrauterine growth retardation, and recurrent wheezing (defined as ≥2 episodes of wheezing prior to admission). Maternal diet during pregnancy was assessed as described in our previous assessment of RSV risk factors for severity in the 2011 population.[11] Briefly, we used a food-frequency questionnaire modified from Willett and colleagues[16–18] with a comprehensive food list representative of the eating habits of the population under study, derived from the Argentinian Health and Nutrition Survey for pregnant women, and further categorized based on ingestion of macronutrients. Given that we did not expect significant changes in dietary habits between early and late pregnancy,[19] the reference period included the dietary intake during the last trimester of pregnancy. This questionnaire requested that respondents estimate their daily, weekly, monthly, and rarely/ever consumption frequencies of individual foods (eg, bread).[15] Given the 90-day period of reference and consequent concerns about portion recall, we did not collect daily information about portion sizes.

2.3 | HRV diagnosis and sequencing

Nasopharyngeal aspirates were obtained upon admission from eligible children using a standard procedure in the 12 participating institutions. Briefly, samples were obtained by gently flushing the infants' nostrils with 4 mL of sterile saline solution (AnalytiCals, Chemit, Buenos Aires, Argentina). Secretions were aliquoted (1 mL), and immediately stored in dry ice, and transferred to −80°C until use. HRV was detected in duplicates by real-time reverse transcriptase-polymerase chain reaction with the Smart-CyclerII (Cepheid, Sunnyvale, CA) by using primers and probe sequences directed at a highly conserved HRV 5′-non-coding region.[20] Viral load was determined by the number of amplification cycles needed for a positive PCR test (cycle threshold, CT). Co-infections with RSV, influenza viruses, human metapneumoviruses, and human parainfluenza viruses were ruled out by RT-PCR, as described.[21]

A total of 294/347 (85%) HRV positive specimens were selected for sequencing with the Smart Cycler II (Cepheid) using primers and probe sequences (CY+AGCC+TGCGTGGC, GAAACACGGACACC-CAAAGTA, TCCTCCGGCCCCTGAATGYGGC) directed at a highly conserved HRV 5′-noncoding region.[20] Conventional RT-PCR was then performed by using primers that amplified a fragment of approximately 548 nt, encompassing the VP4/VP2 region, and the hypervariable region in the 5′-NCR.[22] Amplified fragments were

sequenced with the AbiPrism-BigDye-Terminator Kit (Applied-Biosystems, Thermo Fisher Scientific Inc., Pittsburgh, PA) on a 3730xl DNA-Analyzer (Applied-Biosystems). Sequences were edited and aligned with MacVector-version-11.1 (MacVector Inc., Apex, NC). Seven published strains from GenBank and 136 field strains from this study were included. Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA Software & Technologies, Tempe, AZ) with three bootstrapped replicates and the neighbor-joining algorithm with HRV87 as outgroup.[23]

2.4 | Cytokine determinations

Interferon-γ (IFN-γ), IFN- λ_1, interleukin-13 (IL-13), IL-4, IL-5, and IL-9 were evaluated by immunoassay (Becton, Dickinson and Company, Chandler, AZ) in nasopharyngeal samples according to instructions by the manufacturers.

2.5 | Outcome variable

Patients were considered to have life-threatening disease when presenting with O_2 sat ≤87% on admission,[24,25] Oxygen saturation cut-off for extreme severity was selected based on previous publications[24,25] and to identify seriously ill children with hemoglobin saturations clearly below the flat portion of the oxyhemoglobin dissociation curve.[26] In developing country populations, infants meeting clinical criteria for intensive care or mechanical ventilation may have limited or delayed access to this type of support. In addition, duration of hospitalization may be biased by social, non-medical considerations. Therefore, hypoxemia was selected as a preferable outcome rather than intensive care admission, ventilator support and/or duration of hospital stay.

2.6 | Statistical analysis

Chi-square, analysis of variance (ANOVA) and Student's *t*-test were used to compare characteristics of children where appropriate. Candidate socioeconomic, pregnancy, and infant covariates for assessment were selected a priori (precarious house [tin/mud, dirt floor, no sewage], smoking at home, malnutrition, crowding, smoking during pregnancy, carbohydrates intake, maternal/paternal asthma, age, sex, birth weight, prematurity, intrauterine growth retardation, breastfeeding, underlying chronic illness). Multivariable logistic regression analysis identified risk factors for life-threatening disease among children hospitalized with severe HRV. A *P*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using Stata-12 (Statacorp LLC, College Station, TX).

3 | RESULTS

3.1 | Study population

During the 2011–2013 winter respiratory seasons, 5012 infants and children younger than age 2 years presenting with severe ARI were enrolled in the study. Among hospitalized patients, HRV was detected as a single pathogen in 347 patients, 118 (34.0%) in 2011, 132 (38.0%) in 2012, and 97 (28.0%) in 2013. RSV was the main pathogen identified during the 3 years.

The 347 hospitalized patients with HRV single-pathogen infections were included in our study. Among the 347 patients identified with HRV as a single pathogen, 315 (90.8%) presented with severe illness (defined as an O_2 sat between 88 and 92% when breathing room air) while 32 (9.2%) patients with life-threatening disease (defined as an O_2 sat ≤87% when breathing room air) were the main subjects of our investigation.

3.2 | Epidemiological and clinical risk factors for life threatening HRV infection

To characterize risk factors affecting extreme severity of illness in HRV infection, we first investigated whether variables reflecting the socioeconomic status of families, maternal habits during pregnancy, and clinical characteristics of infants and young children were associated with life-threatening disease (Table 1). Given the association between HRV and subsequent frequent wheezing in children[7] and between HRV and underlying chronic lung disease in premature infants,[6] we were particularly interested in exploring, in addition to traditional risk factors for acute lung illness,[27] variables potentially associated with lung development and/or a family history of asthma. Among all variables explored (univariate analysis), a high maternal intake of carbohydrates during pregnancy ($P = 0.02$) and recurrent wheezing prior to admission ($P = 0.05$) were associated with life-threatening HRV infection (Table 2). No role in life-threatening disease was identified for other risk factors typically associated with bronchiolitis (Table 2).

3.3 | Role of HRV load and species in disease severity

We subsequently investigated whether HRV viral load in the respiratory tract or HRV group associated with life-threatening presentations (Figure 1). A total of 294 (85%) patients had NP samples available for typing. HRV-A was responsible for 31.6%, HRV-B for 3.7% and HRV-C for 19.1% of infections (Figure 1A-D). Our failure rate for HRV typing was 44.9% (Figures 1A and 1C). Of all episodes attributable to HRV, 1.9% of HRV-A, 0.97% of HRV-B, 2.9% of HRV-C, and 4.8% non-typable-HRV were life-threatening (Figure 1A; $P = 0.2$ between groups).

Interestingly, HRV viral load did not differ between life-threatening and severe, non-life threatening infections ($P = 0.72$; Figure 1B). In fact, an exploratory comparison of viral load between life-threatening and severe cases elicited by individual HRV groups also failed to demonstrate differences (P = NS for all three comparisons; Figure 1C). Moreover, no differences were observed when comparing HRV viral load between A and C groups ($P = 0.38$). In fact, comparisons of individual clinical signs including cough, wheezing, tachypnea, rhinorrhea, and fever did not differ between HRV groups (not shown).

TABLE 1 Epidemiologic characteristics of the population

	All rhinovirus + patients *n* = 347	Severe disease O_2 saturation 88-93% *n* = 315 (90.78%)	Life-threatening disease O_2 saturation <87% *n* = 32 (9.22%)
Socioeconomic variables			
Precarious home[a] (*n*,%)	298	267 (89.6)	31 (10.4)
Smoking at home (*n*, %)	215	196 (91.16)	19 (8.84)
Maternal education[b] (*n*, %)	52	44 (84.62)	8 (15.38)
Crowding (*n*, %)	173	159 (91.90)	14 (8.09)
Familial variables			
Parental asthma (*n*, %)	40	34 (85)	6 (15)
Sibling atopy (*n*, %)	31	27 (87.10)	4 (12.90)
Pregnancy variables			
Smoking during pregnancy (*n*, %)	62	56 (90.32)	6 (9.68)
Intrauterine growth retardation (*n*, %)	21	20 (95.24)	1 (4.76)
Carbohydrate intake[c] score (mean, CI 95%)	19.08 (18.67-19.5)	18.54 (17.48-19.6)	20 (15.03-24.97)
Infant variables			
Age, months (mean, range)	4.11 (0-20)	4.22 (0-23)	5.18 (0-10)
Birth weight in grams (mean, range)	3071 (800-4800)	3161 (800-4800)	3071 (800-4500)
Prematurity (*n*, %)	56	49 (87.5)	7 (12.5)
Male (*n*, %)	197	183 (92.89)	14 (7.11)
Breastfeeding (*n*, %)	209	193 (92.34)	16 (7.66)
Underlying chronic illness[d] (*n*, %)	34	27 (79.42)	7 (20.58)
Recurrent wheezing	81	69 (85.19)	12 (14.81)
Malnutrition[e] (*n*, %)	10	9 (90)	1 (10)
Laboratory variables			
IL 13, pg/mL (mean, range)	4.93 (0.001-31.33)	3.42 (0.001-31.33)	17.31 (0.001-27.83)
IFNλ_1, pg/mL (mean, range)	8.98 (0.001-96.3)	9.15 (0.001-96.3)	2.55 (0.001-43.35)

[a]Precarious home: House material tin/mud; dirt floor; no sewage; heating unvented sources; lack of potable water.
[b]Maternal education: Incomplete primary school.
[c]Carbohydrate rich food groups: Bread, pastries, sugar sweetened beverages, sweetened infusions, pasta & rice, and potatoes.
[d]Immunodeficiency, congenital hearth disease, or neurological disorder.
[e]Malnutrition: % of the infant's weight compared to that of a normal child (50th percentile of weight for age) of the same age under 90% according to the World Health Organization child growth standards: http://www.who.int/childgrowth/standards/en/

In summary, our evaluations did not detect a significant role for specific HRV groups or HRV viral load in association with life-threatening disease.

3.4 | High interleukin-13 in the respiratory tract and life-threatening HRV illness

Then, we investigated whether cytokines previously associated with HRV-mediated wheezing in older patients[8,10] affected severity of acute lung disease in young infants and children, focusing on Th cytokines and IFNλ_1 production (Figure 2). We therefore investigated the Th-bias of life-threatening presentations by comparing IFN-γ and IL-13 responses in respiratory secretions (Figures 2A and 2B). In this case, higher IL-13 Levels associated with life-threatening HRV infections (OR = 1.06 [95%CI, 1.04-1.1]; $P < 0.001$; $P = 0.0083$ using Bonferroni, Figure 2B). In fact, IL-13 Levels >10 pg/mL in respiratory secretions on presentation strongly associated with life-threatening disease (OR = 7.60 [95%CI, 3.19-18.10]; $P < 0.001$). Moreover, IL-13 Levels correlated with SpO2 on admission ($P = 0.0013$; Figure 3). Conversely, no association was detected between severity of illness and IFN-γ levels in the respiratory tract (Figure 2A).

We further investigated the role of IL-13 in severity associated with specific HRV groups (Supplementary Figure S1). While IL-13 Levels exhibited a trend toward higher concentrations of the cytokine in life threatening cases only for HRV-C, none of these comparisons reached statistical significance.

Given the risk for life threatening HRV illness in children with recurrent wheezing and in those with higher levels of IL-13 in the respiratory tract, we speculated that other Th2 cytokines would also increase in association with severity (Figure 2D-F). Interestingly, no differences were observed in IL-4, IL-5, or IL-9 Levels between infants and children presenting life-threatening versus severe disease (Figure 2D-F). Levels of IFNλ_1 did not associate with life-threatening HRV disease (OR = 0.96 [0.92-1.01]; $P = 0.138$; Figure 2C).

TABLE 2 Risk factors for life-threatening disease in children hospitalized with HRV infection

	Univariate analysis	
	Life-threatening disease O_2 saturation <87% *n* = 32 (9.22%) OR (CI 95%)	***P*-value**
Socioeconomic variables		
Precarious home[a]	1.06 (0.44–2.56)	0.9
Smoking at home	0.84 (0.40–1.77)	0.65
Maternal education[b]	1.66 (0.68–4.1)	0.27
Crowding	0.77 (0.37–1.61)	0.49
Familial variables		
Mother asthma	1.03 (0.84–6.86)	0.87
Father asthma	2.52 (0.79–8.08)	0.12
Parental asthma	1.69 (0.58–4.95)	0.34
Sibling atopy	1.46 (0.46–4.65)	0.63
Pregnancy variables		
Smoking during pregnancy	1.05 (0.41–2.67)	0.92
Intrauterine growth retardation	0.52 (0.07–4.07)	0.54
Carbohydrate intake[c] score	1.26 (1.03–1.53)	0.02
Infant variables		
Age, months	1.08 (0.96–1.21)	0.22
Birth weight in grams	1 (0.99–1)	0.83
Prematurity	1.51 (0.62–3.71)	0.37
Male	0.52 (0.25–1.09)	0.08
Breastfeeding	1.998 (0.56–7)	0.29
Underlying chronic illness	2.48 (0.93–6.62)	0.07
Recurrent wheezing	2.16 (1.4–4.67)	0.05
Malnutrition[d]	3.11 (0.9–10.8)	0.07
Laboratory variables		
IL13	1.06 (1.04–1.1)	<0.001
IFNλ_1	0.96 (0.92–1.01)	0.14

[a]Precarious home: House material tin/mud; dirt floor; no sewage; heating unvented sources; lack of potable water.
[b]Maternal Studies: Incomplete primary school.
[c]Carbohydrate rich food groups: bread, pastries, sugar sweetened beverages, sweetened infusions, pasta & rice, and potatoes.
[d]Malnutrition: % of the infant's weight compared to that of a normal child (50th percentile of weight for age) of the same age under 90% according to World Health Organization child growth standards: http://www.who.int/childgrowth/standards/en/

3.5 | Multivariable analysis

Finally, we weighted the role of variables found to significantly affect the odds of life threatening HRV presentations in a multivariable analysis. High maternal intake of carbohydrates (OR = 1.28 [1.01-1.6]; *P* = 0.04) during the third trimester of pregnancy and IL-13 Levels (OR = 1.05 [1.01-1.11]; *P* = 0.03] in the respiratory tract affected the odds of life-threatening illness (Table 3). The role of recurrent wheezing illness was not significant in the multivariable model (Table 3). A second multivariable analysis weighting IL-13 >10 pg/mL also exhibited significant odds ratios for life threatening illness for IL-13 Levels (OR = 7.43 [3-18.4]; *P* < 0.001) but not sugar intake during pregnancy (OR = 1.33 (0.45-3.92); *P* = 0.6) and recurrent wheezing.

4 | DISCUSSION

In this study, we evaluated epidemiological, viral, and immunological risk factors for life-threatening illness due to HRV in infants and children younger than 2 years of age. Given the frequent mild presentations of HRV infections in infants, understanding factors that promote or identify the subgroup of young children with extremely severe illness is important. High IL-13 Levels in respiratory secretions during infection predisposed to severe hypoxemia with O_2 sats ≤87% upon admission to the hospital. No role was detected for viral load in the upper respiratory tract, specific viral groups, traditional risk factors for severe disease due to other respiratory viruses (eg, crowding, smoking at home), or other cytokines associated with Th-bias.

The association between IL-13 Levels and life threatening HRV disease is in line with the well-described link between the virus and asthma exacerbations. Prior work described a maternal history of atopy and asthma in association with HRV hospitalization (not necessarily life threatening).[7] Moreover, adult asthmatic volunteers infected with HRV-16 had higher levels of IL-13 in supernatant fluids of bronchoalveolar lavage cells incubated with mitogens when compared to infected non-asthmatic controls.[9] In fact, association between variation at the-17q21-locus and asthma in children who had HRV wheezing illness[28] and our data suggest that infants with life-threatening HRV disease may be experiencing their "first severe asthmatic exacerbation."

Interestingly, higher levels of IL-4 in extremely severe cases did not accompany the raise in IL-13 secretion. This observation raises two important issues. First, unlike for HRV, severe ARI due to RSV in infants associates with high levels of IL-4 but not IL-13.[29,30] Therefore, different cytokine profiles may lead to similar clinical presentations of severe illness caused by different viruses. These differences stress that bedside etiologic tests for viral ARI may become more critical for targeted therapies in the future. Second, increases in other Th2 cytokines would be expected during HRV infection in the context of a classic Th2 response. Therefore, this early IL-13 surge may be the product of IL-13 producing innate lymphoid cells type 2 (ILC-2).[31] ILC-2 are important sources of early IL-13 during pulmonary allergic inflammation.[32] Therefore, they may contribute to the association between a maternal history of atopy and asthma[7] or recurrent wheezing with severe HRV infections. Hence, viruses may mobilize different cytokines from different types of immune cells to elicit clinical presentations that are indistinguishable for pediatricians. Consequently, should these cytokines play a role in virus-specific disease

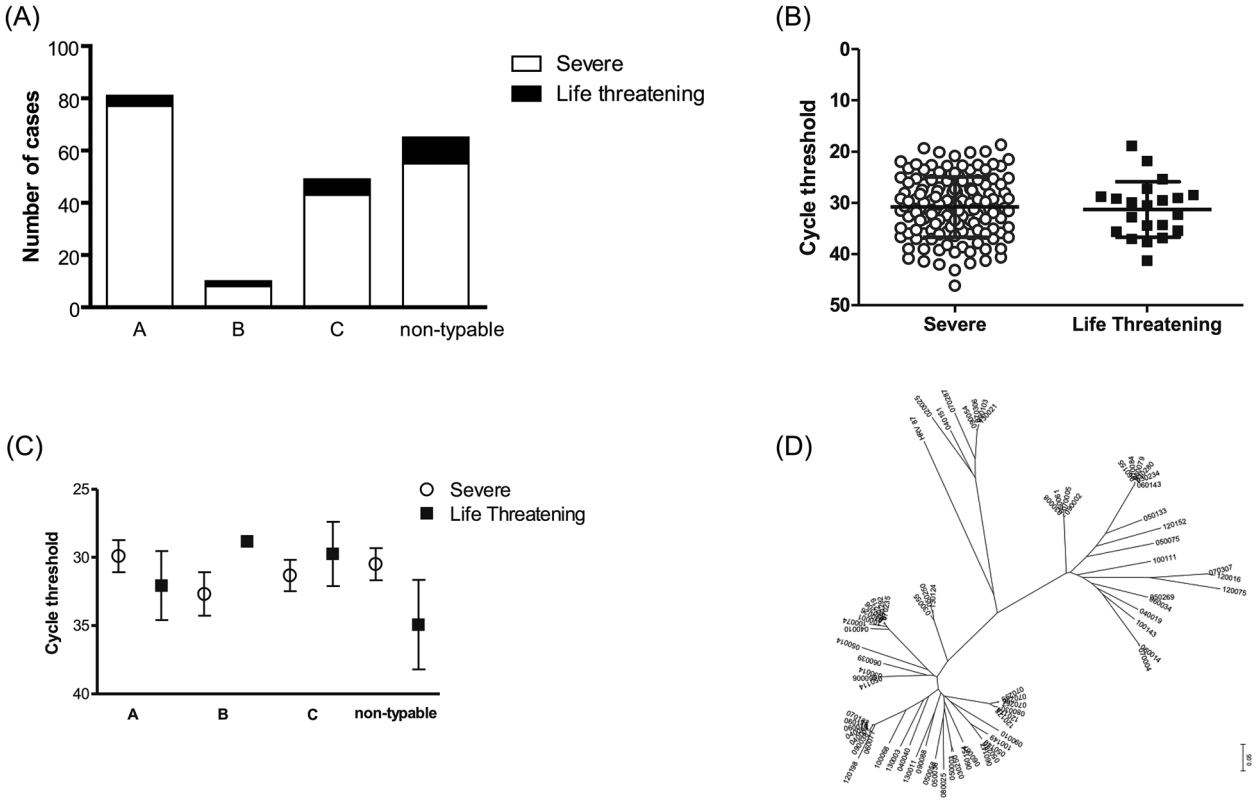

FIGURE 1 Role of HRV species in disease severity. A, Number of cases observed for each species of HRV A, HRV B, HRV C, and those unable to be typed. Four percent of HRV A, 1% of HRV B, 5% of HRV C, and 5% non-typable HRV were life-threatening. B, HRV load in severe compared with life-threatening illness. C, Viral load examined by HRV species. Comparisons between life-threatening and severe presentations. D, Neighbor joining phylogenetic tree of HRV species showing clusters of HRV A, HRV B, and HRV C, with HRV-87 used as outgroup

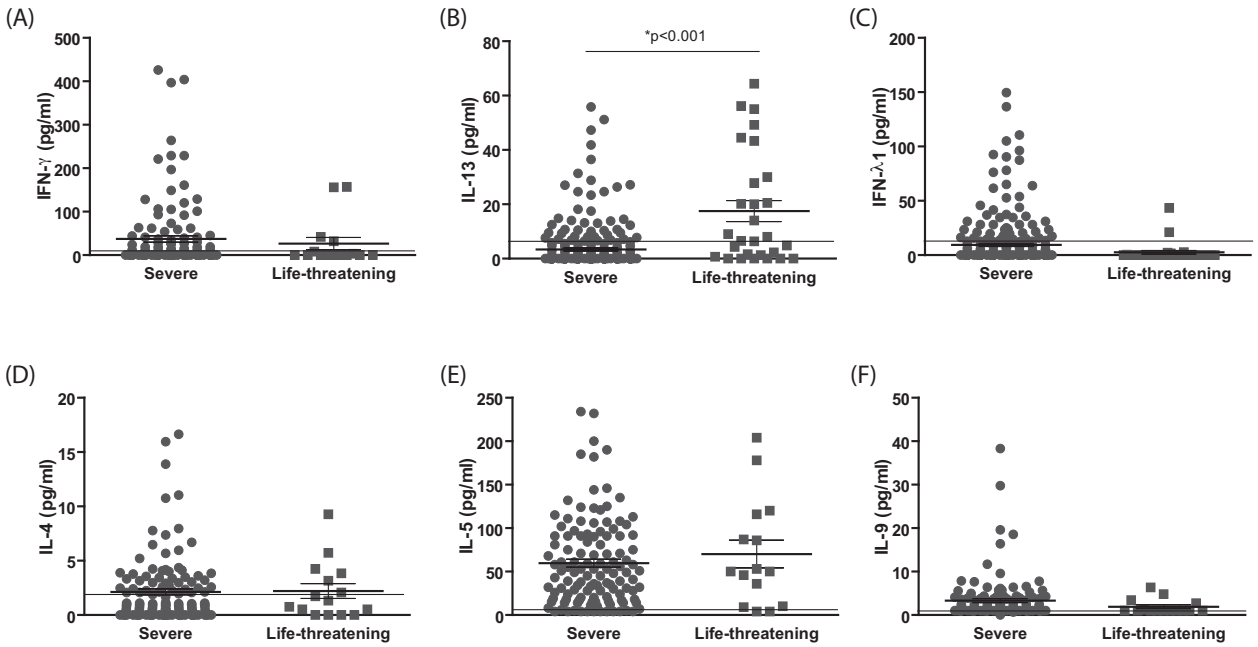

FIGURE 2 Respiratory cytokines in children with life-threatening versus severe HRV disease. A, IFNγ; B, IL-13; C, IFNλ_1; D, IL-4; E, IL-5; and F, IL-9. All values are expressed in pg/mL. Horizontal short lines in panels represent mean values. Solid lines define detection thresholds. There is a significant association between elevated IL-13 Levels and life-threatening disease (OR = 1.07 [1.04-1.1] P = < 0.001). There is no significant relationship between severity and any other tested cytokine

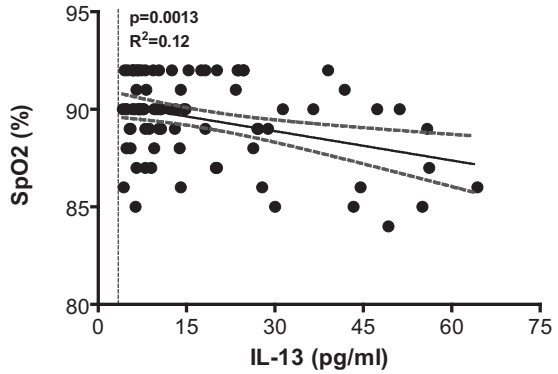

FIGURE 3 Correlation between IL-13 Levels and SpO2 at admission. Scatter plot illustrates linear regression correlation analysis for association between IL-13 Levels (pg/mL and SpO2 (%) at admission for all hospitalized infants with HRV infection (black circles). The R^2 and *P*-value, obtained by Pearson correlation analysis, are displayed on the graphic and 95% confidence intervals were represented with grey dots lines. IL-13 cut off level was 4 pg/mL (black vertical dot line)

pathogenesis, bedside etiologic diagnosis may become invaluable to guide specific treatments.

Unlike other studies, we did not identify a specific HRV group or higher virus load in association with severity. While HRV-C has been the group most commonly associated with severe illness, groups associated with higher risk have varied.[33,34] In fact, other studies like ours, which had a relatively high failure rate for HRV subgrouping-failed to detect similar associations suggesting that other modulatory factors may influence the role of HRV-groups in disease.[35,36] Discrimination of the precise role of specific HRV groups in severity of illness in infants and young children require additional studies.

Our study has limitations. HRV circulates year round and may vary in severity during different seasons of the year.[37] For this reason, we did not assess HRV disease-burden. While we conducted this study in a catchment population of over 50 000 children every year, and included more than 5000 hospitalized patients, the relatively low frequency of severe single-pathogen HRV infections limited our power to further assess variables potentially modulating risk, particularly given our interest in characterizing children severely compromised by the virus. In addition, we obtained respiratory secretions on admission, and cytokine responses may differ at other time points. However, these data at an early time point describe changes in cytokine responses that may therefore be amenable to modulation. Two recent studies have shown that blocking IL-33 in human bronchial epithelial cells infected with HRV or in mice sensitized with OVA and infected with RV reduced IL-13 Levels.[38,39] Assessment of IL-33 Levels in affected infants will be important in the future. Also, we sequenced a representative 85% of HRV isolates but experienced a high failure rate. Finally, although our population reflected infants and young children living in regions of very low socioeconomic status, we did not find a relationship between variables reflecting social vulnerability and life threatening disease due to HRV.

TABLE 3 Multivariable analysis of risk factors for life-threatening disease in children hospitalized with HRV infection

	Multivariable analysis	
	OR (CI 95%)	*P*-value
Recurrent wheezing	0.84 (0.16-4.52)	0.84
Carbohydrate intake[a] score	1.28 (1.01-1.6)	0.04
IL-13	1.05 (1.01-1.11)	0.03

[a]Carbohydrate rich food groups: Bread, pastries, sugar sweetened beverages, sweetened infusions, pasta & rice, and potatoes.

The study also has significant strengths. To our knowledge, ours is the first population-based prospective study to evaluate numerous candidate dependent variables for life-threatening HRV illness in infants and young children, simultaneously analyzing the role of candidate viral and immune determinants of extremely severe disease. In fact, risk factors fit well within previous hypotheses of HRV pathogenesis.[40,41] In line with its leading role in extreme premature babies,[6] HRV was second to RSV in frequency among severe cases from 2011 to 2013, even while only studied during the winter respiratory season. Additionally, none of the patients included in this study developed a severe bacterial co-infection suggesting that mechanisms of HRV disease severity may differ from those driven by RSV or influenza virus.

In summary, HRV can trigger life-threatening respiratory illness in infants and young children. These presentations associate with high early levels of IL-13 in the respiratory tract. Targeted interventions against IL-13 during acute illness may decrease severity of HRV infections in infants. The diverse cytokine profiles associated with ARI severity in different viral infections stresses the need for bedside etiologic diagnoses. In addition, comprehensive programs focused on health and habits of pregnant women and their future babies may impact severity of HRV and other ARI.

5 | INFANT RESPIRATORY NETWORK MEMBERS

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CONFLICTS OF INTEREST

All authors report no conflict of interest for this manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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