Accepted Article

Article Type: Original

Rhinovirus-induced Airway Cytokines and Respiratory Morbidity in Severely Premature Children

Geovanny F. Perez, MD[1-4]; Krishna Pancham, MD[1,2]; Shehlanoor Huseni, MD[1,2]; Amisha Jain, MD[1,2]; Carlos E. Rodriguez-Martinez, MD, MSc [5-7] ; Diego Preciado, MD, PhD[2-4,8]; Mary C. Rose, PhD[1-4,9], Gustavo Nino, MD, MSc [1-4]

Affilations: [1] Division of Pulmonary and Sleep Medicine, Children's National Medical Center, Washington, DC; [2] Department of Pediatrics, George Washington University School of Medicine and Health Sciences, Washington, DC;[3] Department of Integrative Systems Biology, George Washington University, Washington, DC;[4] Center for Genetic Research Medicine, Children's National Medical Center, Washington, DC;[5] Department of Pediatrics, School of Medicine, Universidad Nacional de Colombia, Bogota, Colombia;[6] Department of Pediatric Pulmonology and Pediatric Critical Care Medicine, School of Medicine, Universidad El Bosque, Bogota, Colombia;[7] Research Unit, Military Hospital of Colombia, Bogota, Colombia; [8] Division of Pediatric Otorhinolaryngology, Departments of Surgery and Pediatrics, George Washington University School of Medicine and Health Sciences, Washington, DC; [9] Department of Biochemistry and Molecular Medicine, George Washington University, Washington, DC

Address correspondence to: Gustavo Nino, MD, MSc; Division of Pediatric Pulmonology and Sleep Medicine Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010, gnino@childrensnational.org

Short title: RV-induced Th2/Th17 in Prematurity
Abbreviations: RV – rhinoviurs; BPD– bronchopulmonary dysplasia
Key Words: Rhinovirus, bronchopulmonary dysplasia, prematurity, asthma Th2, Th17
Funding source: This work was supported by Grants NHLBI/HL090020 (K12 Genomics of Lung), NICHC/HD001399 (K12 Child Health Research Career Development Award), UL1TR000075 KL2TR000076 Awards from the NIH National Center for Advancing Translational Sciences
Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.
Conflict of Interest: The authors have no conflicts of interest to disclose.

Contributor's Statements:
Geovanny F. Perez, Gustavo Nino: Drs. Nino and Perez conceptualized and designed the study, coordinated experiments, drafted the initial manuscript, and approved the final manuscript as submitted.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12346
This article is protected by copyright. All rights reserved.

Accepted Article

Krishna Pancham, Shehlanoor Huseni, Amisha Jain: Drs. Pancham, Huseni and Jain carried out specimen collection, clinical data acquisition and coordinated cytokine experiments, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Carlos E. Rodriguez-Martinez, Gustavo Nino: Drs. Rodriguez-Martinez and Nino designed the data collection instruments,conducted statistical analysis, reviewed the manuscript, and approved the final manuscript as submitted.

Diego Preciado, Mary C. Rose: Drs. Rose and Preciado critically reviewed study design, coordinated and supervised data collection, reviewed the manuscript, and approved the final manuscript as submitted.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ABSTRACT

Background

Rhinovirus (RV) has been linked to the pathogenesis of asthma. Prematurity is a risk factor for severe RV infection in early life, but is unknown if RV elicits enhanced pro-asthmatic airway cytokine responses in premature infants. This study investigated if young children born severely premature (<32 weeks gestation) exhibit airway secretion of Th2 and Th17 cytokines during natural RV infections and if RV-induced Th2-Th17 responses are linked to more respiratory morbidity in premature children during the first two years of life.

Methods

We measured Th2 and Th17 nasal airway cytokines in a retrospective cohort of young children aged 0-2 years with PCR-confirmed RV infection or non-detectable virus . Protein levels of IL-4, IL- 13, TSLP and IL-17 were determined with multiplex immunoassays. Demographic and clinical variables were obtained by electronic medical record (EMR) review.

Results

The study comprised 214 children born full term (n=108), pre-term (n=44) or severely premature (n=62). Natural RV infection in severely premature children was associated with elevated airway secretion of Th2 (IL-4 and IL-13) and Th17 (IL-17) cytokines, particularly in subjects with history of bronchopulmonary dysplasia. Severely premature children with high RV-induced airway IL-4 had recurrent respiratory hospitalizations (median 3.65 hosp/year; IQR 2.8-4.8) and were more likely to have at least one pediatric intensive care unit admission during the first two years of life (OR 8.72; 95% CI 1.3-58.7; p=0.02).

Conclusions

Severely premature children have increased airway secretion of Th2 and Th17 cytokines during RV infections, which is associated with more respiratory morbidity in the first two years of life

This article is protected by copyright. All rights reserved.

Accepted Article

INTRODUCTION

Rhinovirus (RV) is the most common cause of asthma exacerbations in children and it is linked to long-term progression of the disease.[1-3] RV infection also causes severe respiratory illnesses in premature infants,[4-7] particularly in those with history of bronchopulmonary dysplasia (BPD).[4-7] Moreover, recent studies have demonstrated that RV is the most common cause of lower respiratory tract infection in premature children during the first two years of life.[7] Cost-economic analyses indicate that RV-induced wheezing episodes in premature infants increase prematurity-related healthcare costs during early childhood,[8] even more so than other viruses like respiratory syncytial virus (RSV).[8] Despite the importance of the clinical link between RV and prematurity, the reason why premature babies are senstitive to RV infections during the first years of life is still largely unknown.

We have recently described that RV infection in young children elicits airway secretion of the master Th2 cytokine thymic stromal lymphopoetin (TSLP) with concomitant Th2 bias in nasal cytokine profiles.[9] These findings are in agreement with animal models in which RV generates strong airway Th2 responses.[10,11] Th17 cytokines (i.e. IL-17) are also known to promote the development of pro-asthmatic changes in the airways,[11] and regulate airway inflammation during viral respiratory infections.[12-14] Interestingly, it is unknown if naturally occurring RV infections elicit pro-asthmatic airway cytokine responses (Th2-Th17) in premature babies during early infancy. To address this critical gap in our knowledge, we measured Th2 and Th17 cytokines levels in the nasal secretions of a retrospective cohort of young children (n=214) with PCR-confirmed RV infection or non-detectable virus (control). Our aim was to investigate

This article is protected by copyright. All rights reserved.

Accepted Article

if young children prematurily born exhibit enhanced nasal airway secretion of Th2 and Th17 cytokines during natural RV infections in early life compared to children born pre-term or full-term. Secondary analysis examined if the presence of RV-induced Th2 and Th17 airway profile in severely premature children is linked to a history of BPD, recurrent respiratory hospitalizations and need for admission to the pediatric intensive care unit (PICU) during the first two years of life.

METHODS

Study population

This is a retrospective cohort study designed to contrast nasal airway cytokine responses during rhinovirus (RV) infection in premature and non-premature young children. We identified 264 patients aged 0-2 years with PCR confirmed RV infection (n=166) or non-detectable virus by PCR testing (controls,n=98) during the study period (February 2013 to June 2014) in an available electronic database and biorepository of children undergoing nasal lavage for diagnostic purposes (respiratory virus detection by PCR) available at Children's National Medical Center. Subjects with mixed viral infections or insufficient nasal sample for cytokine analysis were excluded (n=50). For the purpose of the study "severe prematurity" was defined by a gestational age (GA) of less than 32 weeks to include extremely preterm and very preterm subjects, "preterm" as 32-37 weeks GA and "full-term" as >37 weeks GA according to WHO definitions.[15,16] RV and negative virus statuses were confirmed by PCR analysis used for clinical purposes in our institution. Clinical and demographic variables were obtained by reviewing

This article is protected by copyright. All rights reserved.

electronic medical records (EMR). This study was approved by the Institutional Review Board of Children's National Medical Center, Washington D.C.

Nasal washing collection, viral PCR analysis and cytokine measurements

Nasal airway secretions were collected at the onset of acute respiratory illnesses by a standard nasal lavage technique consisting of gently washing the nasal cavity with 3-4 mL sterile normal saline. Secretions were aliquoted in 3 separate ependorf tubes to avoid tawn and untawn samples and stored at -80^{o}C until further analysis. Nasal samples were analyzed by a viral multiplex PCR panel for 12 targets (rhinovirus, RSV A, RSV B, HMPV, parainfluenza 1-3, influenza A and B, H1N1, H1N3, Adenovirus) used for clinical purposes (Luminex, TX, USA) according to the microbiology laboratory protocol of our institution. Nasal washings were analyzed for protein levels of TSLP, IL-13, IL-4 and IL-17 using a commercially available multiplex magnetic bead immunoassay (Millipore, MA, USA) according to the manufacturers' instructions using provided standards and quality controls.

Clinical and demographic variables

Clinical and demographic variables were obtained by EMR review at Children's National Medical Center. Demographic variables were obtained in all study subjects (n=214) and comprised gestational age in weeks, age, gender and ethnicity. Sub-analysis of severely premature children (<32 weeks GA) with at least 6 months of EMR at the end of the study period and complete neonatal/perinatal history (n=45) included clinical binary variables such as family

This article is protected by copyright. All rights reserved.

Accepted Article

history of asthma and history of early neonatal/perinatal events such as necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), need for endotracheal intubation plus mechanical support (for at least 72hr) in the neonatal intensive care unit (NICU) and bronchopulmonary dysplasia (BPD). For the purpose of this investigation BPD was determined using the definition of the National Heart, Lung and Blood Institute (NHLBI).[17] In this sub-group of prematurely born children we also used EMR data obtained during the first two years of life to calculate the annualized number of hospital admissions due to respiratory concerns (respiratory hospitalizations/year) and the binary presence of at least one admission to the intensive care unit (PICU) due to respiratory distress during the first two years of life.

Statistical analysis

Data were analyzed using the software SAS version 9.3 or later (SAS Institute Inc., Cary, NC, USA). Data within each group (RV-infected and control) were analyzed with two sample t-test or Wilcoxon rank-sum for continuous variables and $\chi 2$ test for binary variables. Cytokine levels comparisons between full-term, pre-term and severly premature groups were performed with non-parametric ANOVA (Kruskal-Wallis) with post-test analysis. Multivariable linear and logistic regression models were built to study the link between clinical variables (i.e. BPD and respiratory hospitalizations) and Th2 and Th17 airway inflammatory responses adjusted by pertinent covariables. A probability of <0.05 was considered statistically significant.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

RESULTS

Baseline Characteristics

Of the 264 initially identified children, 50 were excluded due to the presence of mixed viral infection.A total of 214 patients were included in this study, 116 patients had positive PCR for RV and 98 patients had negative viral PCR testing (control group). There were not significant differences in the baseline demographic characteristics of the RV and control groups, including gender, age, ethnicity and gestational age (Table 1). To contrast Th2 and Th17 airway cytokine profiles in premature vs. non-premature children, RV and control groups were subdivided into three separate categories, severely premature (<32 weeks GA; n=62), preterm (32-37 weeks GA; n=44) and full-term (>37 weeks GA; n=108) (Figure 1).

Rhinovirus-induced Th2 and Th17 nasal airway cytokines in premature and full-term children

Our results identified that young children with history of severe prematurity and naturally occurring RV infection had significantly elevated nasal airway protein levels of classical Th2 cytokines (IL-4 and IL-13) compared to severe premature children without detectable virus (IL-4: control severe premature 2.9 pg/ml, IQR [interquartile range] 1.7-5.2 vs. RV severe premature 6.42 pg/ml, IQR 4.4-14.5; IL-13: control severe premature 2.26 pg/ml, IQR 1.4-3.9 vs. RV severe premature 5.15 pg/ml, IQR 3-11.4, Figure 2 A-B), however, not significant differences were identified among preterm and full-term subjects (Figure 2 A-B). TSLP nasal protein levels were also elevated in premature children with RV infection compared

This article is protected by copyright. All rights reserved.

to premature subjects without any identifiable virus (control severe premature 9.8 pg/ml, IQR 1.2-20 vs. RV severe premature 19.1 pg/ml, IQR 14.5-31; Figure 2C), but this TSLP secretion was equally elevated in preterm and full-term children with RV infection (Figure 2C). To explore if acute RV infection was also linked to enhanced nasal secretion of Th17 cytokines we measured protein levels of IL-17 in the same study subjects. As shown in Figure 2D young severely premature children with RV infection had significantly elevated IL-17 protein levels relative to severely premature children without identifiable virus (control severe premature 1.5 pg/ml, IQR 0.5-3.3 vs. RV severe premature 3.8 pg/ml, IQR 2.2-10.9; Figure 2D) but no significant differences were found in RV-infected and control preterm and full-term subjects (Figure 2D).

Effect of bronchopulmonary dysplasia (BPD) in rhinovirus-induced Th2 and Th17 nasal airway cytokines in severely premature children

We next conducted a sub-analysis of the data from the group of severely premature children using relevant clinical variables. Only subjects with at least 6 months of EMR at the end of the study period and complete neonatal/perinatal history were included (n=45). As shown in Table 2, initial analysis revealed that control and RV-infected severely premature children had comparable baseline characteristics, including demographics, GA and the proportion of children with family history of asthma, BPD, NICU mechanical ventilation, NEC or IVH (Table 2). To study the effect of BPD in rhinovirus-induced Th2 andTh17 responses we contrasted nasal protein levels of IL-4 and IL-17 in severely premature children with and without history of BPD (n=45, Fig 3). RV infection was associated with significantly higher nasal protein levels of IL-4

This article is protected by copyright. All rights reserved.

Accepted Article

and IL-17 in severely premature children with history of BPD (IL-4: control with BPD 1.9pg/ml, IQR 1.4-3 vs. RV with BPD 9.9pg/ml, IQR 6.7-18; IL-17: control with BPD 1.2pg/ml, IQR 0.4-2.3 vs. RV with BPD 4.8pg/ml, IQR 2.4-1.2; Figure 3) but not in the severely premature children without a history of BPD (IL-4: control without BPD 2.2pg/ml, IQR 1.4-4 vs. RV without BPD 3.2pg/ml, IQR 2.2-5.3; IL-17: control without BPD 1.1pg/ml, IQR 0.5-3 vs. RV without BPD 2pg/ml, IQR 0.6-2.3; Figure 3). Multivariate linear regression models identified that the association between BPD and higher IL-4 and IL-17 levels in severely premature subjects with RV infection was independent of other prematurity related variables (BPD, NEC, IVH, Family history of asthma, NICU mechanical ventilation; Table 3).

Multivariate analysis of rhinovirus-induced IL-4 and IL-17 in severely premature children and respiratory hospitalizations

We next explored the clinical significance of the RV induced-Th2 and Th17 airway inflammatory responses in premature children. We first determined the distribution and quartiles (Q) of IL-4 and IL-17 in the entire dataset of RV-infected children (n=116). High levels of IL-4 and IL-17 in RV infected children were defined as values $\geq Q_3$ (3^{rd} quartile). A Q_3 cutoff value of 8.1 pg/ml was selected for high RV-induced IL-4 and 4.6 pg/ml for high RV-induced IL-17. Multivariate analysis (logistic regression) identified that premature children with high nasal IL-4 levels during natural RV infection were associated with 8 times increased odds of having at least one PICU admission during the first two years of life (adjusted OR 8.72; 95% CI 1.3-58.7; p=0.02; Table 4). High RV-induced IL-17 was also associated with 5 times increased odds of history of PICU admission during early childhood (adjusted OR 5.22; 95% CI 1.1-24.5; p=0.03;

This article is protected by copyright. All rights reserved.

Accepted Article

Table 4). Premature children with high RV induced-IL-4, and those with positive family history of asthma, also had more annualized respiratory hospitalizations during the first two years of life independently of gender and race (Table 5). The association between IL-17 and annualized respiratory hospitalizations did not reach statistical significance (Table 5). Neither a history of mechanical ventilation in NICU or BPD alone were significantly associated with more annualized respiratory hospitalizations (Table5).

DISCUSSION

To the best of our knowledge, this is the first study to report that naturally occurring rhinovirus (RV) infection in severely premature children (0-2 years of age) is associated with enhanced nasal airway secretion of Th2 (IL-4 and IL-13) and Th17 (IL-17) cytokines. Furthermore, RV-induced Th2 and Th17 cytokine secretion was linked to bronchopulmonary dysplasia (BPD) and a more severe respiratory phenotype characterized by recurrent respiratory hospitalizations and at least one admission to pediatric intensive care unit (PICU) during the first two years of life. Collectively, our results suggest that premature children have dysregulated Th2 andTh17 airway immune responses to RV infection and provides novel insights about the potential reason why premature babies are sensitive to RV infections during early life.

We recently identified that RV infection in young children elicits nasal airway secretion of the master Th2 cytokine thymic stromal lymphopoeitin (TSLP). [9] In our current study, we also found that nasal TSLP is increased during RV infection, however, this TSLP secretion was equally elevated in premature and full-term children. In contrast, classic Th2 cytokines (IL-4 and IL-13) were significantly higher in the nasal secretions of RV-infected young children with

This article is protected by copyright. All rights reserved.

Accepted Article

history of severe prematurity relative to preterm and full-term subjects. These findings are in agreement with the Th2 airway immune response observed in experimental neonatal RV infection.[10] Using this animal model, Hong et al. demonstrated that neonatal mice infected with RV exhibit significantly increased levels of lung IL-13 relative to older animals, suggesting a strong maturational effect on the link between RV and Th2 responses.[11] The latter may explain, at least in part, why RV-elicits Th2 responses in premature but not in full-term babies. In addition to Th2 responses, we identified that severely premature children exhibit high levels of IL-17 during RV infection. The relevance of this finding is that IL-17 is an important pro-asthmatic cytokine[12] that modulates human airway epithelial responses to RV,[14] which may promote recruitment of neutrophils, immature dendritic cells, and memory T cells in the airways.[13,14] Future translational studies are needed to systematically investigate the exact cellular and molecular consequences of the RV-induced Th2 and Th17 airway responses seen in premature young children.

To determine the early factors associated with the presence of RV-induced Th2 and Th17 airway responses in prematurity, we reviewed electronic medical records (EMR) of 45 severely premature children. This secondary analysis identified that BPD is positively correlated with higher levels of the pro-asthmatic cytokines IL-4 and IL-17 independently of other prematurity-related variables such as necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH) and endotracheal intubation/mechanical ventilation in the neonatal intensive care unit (NICU). RV-induced airway secretion of Th2 andTh17 cytokines was also independent of family history of asthma. Due to incomplete EMR documentation we could not investigate the role of other crucial perinatal/neonatal factors such as chorioamnionitis and breastfeeding, which have been linked to

This article is protected by copyright. All rights reserved.

Accepted Article

BPD and severe RV infections respectively.[7,18,19] It is noteworthy that while the association between BPD and severe RV infection has been described, [7] the potential link between BPD and RV-induced pro-asthmatic cytokine responses in prematurity has not been well elucidated. Interestingly, a recent study identified that prematurity is associated with a 5-fold increased odds of developing asthma in atopic children (but not in non-atopic subjects),[20] suggesting a link between prematurity and the Th2/atopic state. Given that RV promotes Th2 airway responses,[9,10,21] we believe that it is possible that RV and prematurity have additive effects in generating airway pro-asthmatic cytokine responses in children. Longitudinal studies are needed to investigate this postulate and to establish if BPD increases the susceptibility to RV infection during infancy and the risk for asthma and atopy later in life.

To examine the clinical relevance of our findings, we built multivariate models to determine if having high RV-induced IL-4 or IL-17 nasal airway levels (defined as a value $\geq 3^{rd}$ quartile based on data from the entire RV-infected group) is associated with a more severe respiratory phenotype in the first two years of life. Importantly, this analysis identified that premature children with high airway IL-4 or IL-17 during RV infection had nearly 8 times higher odds of having at least one PICU admission due to respiratory distress in the first two years of life (Table 4). Interestingly, family history of asthma was linked to more annualized respiratory hospitalizations in premature children with RV infection. This finding suggests that the pro-asthmatic effect of RV in prematurity may be modulated by genetic factors. This notion is supported by data from the Childhood Origins of Asthma (COAST) and the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) birth cohorts,[3] which identified variants at the 17q21 locus associated with asthma in children who had RV wheezing illnesses and

This article is protected by copyright. All rights reserved.

Accepted Article

expression of two genes at this locus.[3] Further studies are required to delineate the role of these genetic factors in the airway Th2 responses elicited by RV in premature young children.

The high hospitalization rates observed among severely premature infants infected with RV are in accordance with previous reports showing the substantial effect of RV infections in the health-related costs of care of this population.[7,8] Even though this study was not designed to evaluate the economic impact of RV in premature subjects, the fact that premature children with high RV-induced IL-4 or IL-17 airway levels had significantly more annualized respiratory hospitalizations, as well as higher odds of admission to PICU, implies greater health care costs among this group of children. In this regard, it is noteworthy that Drysdale et al recently showed that prematurely born infants with a history of RV lower respiratory tract infection (LRTI) underwent an overall increase in healthcare utilization during infancy and had greater health-related costs of care.[8] In that study the mean cost difference was 1086 GBP (GBP, British pound sterling=1627.86 American dollars) between infants who had at least one LRTI from which RV was detected (by nasopharyngeal apirate) and premature infants who never had a symptomatic LRTI.[8] These increased health costs in the RV group were largely attributed to a greater number of medical visits and persistent airway reactivity (wheezing) at follow up.[8] The latter suggests that the pro-asthmatic effect of RV in the airways, which is potentially linked to enhanced airway Th2 responses,[9-11] may play a key role in the respiratory morbidity and increased health-related costs of care observed in prematurely born children.

Limitations of the present study include the retrospective collection of clinical data and the use of nasal airway samples instead of lung lavages to evaluate the link between RV,

This article is protected by copyright. All rights reserved.

Accepted Article

prematurity and BPD. With respect to the first point, because the data were taken from EMR, and the key variables analyzed are hard variables (i.e. gestational age, viral PCR-result), it is unlikely that its retrospective collection significantly compromised the validity of the results due to misclassifications of disease status (i.e. prematurity or RV infected) or major end-points (i.e. PICU and hospital admissions). However, we recognize that investigating other important variables such as breastfeeding, chorioamnionitis, and clinical severity of RV infections (i.e. wheezing/distress severity scores) may require longitudinal collection of data. In addition we were unable to track the exact time of nasal washing sampling and days of symptoms, which could have played a role in the cytokine protein profile during RV infection. Second, we used nasal samples because it is very difficult to recruit a relatively large population of neonates, infants and young children (n=214 in our study) for a research project involving invasive procedures. Specifically, bronchoalveolar lavage collection via bronchoscopy in young premature children would require anesthesia and endotracheal intubation during acute episodes of lower respiratory infections, a situation in which bronchial edema/secretions and bronchoconstriction may significantly increase the risk for complications. Moreover, nasal samples have been already used by our group and other research teams as a non-invasive surrogate in the evaluation of airway cytokine responses in children during naturally occurring viral infections.[9,22,23]

CONCLUSION

In summary, this study identified that natural RV infection in severely premature young children elicits airway secretion of Th2 (IL-4 and IL-13) and Th17 (IL-17) cytokines. This RV-

This article is protected by copyright. All rights reserved.

Accepted Article

induced Th2 and Th17 cytokines were associated with a history of BPD and more respiratory morbidity during first two years of life due to recurrent respiratory hospitalizations and need for admission to pediatric intensive care unit (PICU). Our data suggest that prematurity is associated with a dysfunctional RV-induced airway immune responses beyond the early neonatal stage, which may underlie why premature children are more sensitive to RV infections during early childhood.

Acknowledgements

Thank you to Karuna Panchapakesan and all the other members of the Research Core Facilities at the Center for Genetic Research Medicine (GenMed) in the Children's Research Institute (CRI) of Children's National Medical Center, Washington, D.C. for their technical support and expertise required for all the airway cytokine measurments presented in this manuscript.

.

REFERENCES

1. Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Gern JE. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol. 2005 Sep;116(3):571-7.
2. Jackson DJ1, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med. 2008 Oct 1;178(7):667-72. doi: 10.1164/rccm.200802-309OC. Epub 2008 Jun 19.
3. Calışkan M, Bochkov YA, Kreiner-Møller E, Bønnelykke K, Stein MM, Du G, Bisgaard H, Jackson DJ, Gern JE, Lemanske RF Jr, Nicolae DL, Ober C. Rhinovirus wheezing illness and genetic risk of

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

childhood-onset asthma. N Engl J Med. 2013 Apr 11;368(15):1398-407. doi:10.1056/NEJMoa1211592. Epub 2013 Mar 27. PMID: 23534543

4. Chidekel AS, Rosen CL, Bazzy AR. Rhinovirus infection associated with serious lower respiratory illness in patients with bronchopulmonary dysplasia. Pediatr Infect Dis J. 1997 Jan;16(1):43-7. PMID: 9002100
5. van Piggelen RO, van Loon AM, Krediet TG, Verboon-Maciolek MA. Human rhinovirus causes severe infection in preterm infants. Pediatr Infect Dis J. 2010 Apr;29(4):364-5. doi:10.1097/INF.0b013e3181c6e60f. PMID: 19935443
6. Costa LF, Queiróz DA, Lopes da Silveira H, Bernardino Neto M, de Paula NT, Oliveira TF, Tolardo AL, Yokosawa J. Human rhinovirus and disease severity in children. Pediatrics. 2014 Feb;133(2):e312-21. doi: 10.1542/peds.2013-2216. Epub 2014 Jan 13. PMID: 24420808
7. Miller EK, Bugna J, Libster R, Shepherd BE, Scalzo PM, Acosta PL, Hijano D, Reynoso N, Batalle JP, Coviello S, Klein MI, Bauer G, Benitez A, Kleeberger SR, Polack FP. Human rhinoviruses in severe respiratory disease in very low birth weight infants. Pediatrics. 2012 Jan;129(1):e60-7. doi: 10.1542/peds.2011-0583. Epub 2011 Dec 26
8. Drysdale SB, Alcazar-Paris M, Wilson T, Smith M, Zuckerman M, Broughton S, Rafferty GF, Peacock JL, Johnston SL, Greenough A. Rhinovirus infection and healthcare utilisation in prematurely born infants. Eur Respir J. 2013 Oct;42(4):1029-36. doi: 10.1183/09031936.00109012. Epub 2013 Apr 5. PMID: 23563263
9. Perez GF, Pancham K, Huseni S, Preciado D, Freishtat RJ, Colberg-Poley AM, Hoffman EP, Rose MC, Nino G. Rhinovirus infection in young children is associated with elevated airway TSLP levels. Eur Respir J. 2014 Jun 25. pii: erj00492-2014.
10. Schneider D, Hong JY, Popova AP, Bowman ER, Linn MJ, McLean AM, Zhao Y, Sonstein J, Bentley JK, Weinberg JB, Lukacs NW, Curtis JL, Sajjan US, Hershenson MB. Neonatal rhinovirus infection induces mucous metaplasia and airways hyperresponsiveness. J Immunol. 2012 Mar 15;188(6):2894-904. doi: 10.4049/jimmunol.1101391. Epub 2012 Feb 13
11. Hong JY, Bentley JK, Chung Y, Lei J, Steenrod JM, Chen Q, Sajjan US, Hershenson MB. Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells. J Allergy Clin Immunol. 2014 Aug;134(2):429-39. doi: 10.1016/j.jaci.2014.04.020. Epub 2014 Jun 6. PMID: 24910174
12. Chesné J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A. IL-17 in Severe Asthma: Where Do We Stand? Am J Respir Crit Care Med. 2014 Aug 27

This article is protected by copyright. All rights reserved.

Accepted Article

13. Stoppelenburg AJ, de Roock S, Hennus MP, Bont L, Boes M. Elevated Th17 response in infants undergoing respiratory viral infection. Am J Pathol. 2014 May;184(5):1274-9. doi: 10.1016/j.ajpath.2014.01.033. Epub 2014 Mar 17.PMID: 24650560

14. Wiehler S, Proud D. Interleukin-17A modulates human airway epithelial responses to human rhinovirus infection. Am J Physiol Lung Cell Mol Physiol. 2007 Aug;293(2):L505-15. Epub 2007 Jun1. PMID: 17545490

15. World Health Organization. 2013. http://www.who.int/mediacentre/factsheets/fs363/en/

16. Blencowe H, Cousens S, Oestergaard M, Chou D, Moller AB, Narwal R, Adler A, Garcia CV, Rohde S, Say L, Lawn JE. National, regional and worldwide estimates of preterm birth. The Lancet, June 2012. 9;379(9832):2162-72.

17. Jobe AH1, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001. Jun;163(7):1723-9.

18. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics. 1996;97:210–215

19. Jobe AH. Effects of chorioamnionitis on the fetal lung. Clin Perinatol. 2012 Sep;39(3):441-57. doi: 10.1016/j.clp.2012.06.010.

20. Rosas-Salazar C, Ramratnam SK, Brehm JM, Han YY, Boutaoui N, Forno E, Acosta-Pérez E, Alvarez M, Colón-Semidey A, Canino G, Celedón JC. Prematurity, atopy, and childhood asthma in Puerto Ricans. J Allergy Clin Immunol. 2014 Feb;133(2):357-62. doi: 10.1016/j.jaci.2013.09.003. Epub 2013 Oct 17. PMID: 24139607

21. Chung Y, Hong JY, Lei J, Chen Q, Bentley JK, Hershenson MB. Rhinovirus Infection Induces IL-13 Production from CD11b-positive, Exudative M2-polarized Exudative Macrophages. Am J Respir Cell Mol Biol. 2014 Jul 16

22. Melendi GA, Laham FR, Monsalvo AC, Casellas JM, Israele V, Polack NR, Kleeberger SR, Polack FP. Cytokine profiles in the respiratory tract during primary infection with human metapneumovirus, respiratory syncytial virus, or influenza virus in infants. Pediatrics. 2007 Aug;120(2):e410-5.

23. Lewis TC, Henderson TA, Carpenter AR, Ramirez IA, McHenry CL, Goldsmith AM, Ren X, Mentz GB, Mukherjee B, Robins TG, Joiner TA, Mohammad LS, Nguyen ER, Burns MA, Burke DT, Hershenson MB. Nasal cytokine responses to natural colds in asthmatic children. Clin Exp Allergy. 2012 Dec;42(12):1734-44. doi: 10.1111/cea.12005.

This article is protected by copyright. All rights reserved.

TABLE 1 .Baseline characteristics for subjects

	Control n=98	Rhinovirus n=116	p-value
Male, n (%)	66(67)	72(63)	0.92
Age (years), median (IQR)	0.73 (0.27-1.18)	0.78 (0.44-1.25)	0.11
Black, n (%)	41(42)	39(48)	0.51
Gestational age (weeks), median (IQR)	37 (27-39)	38 (29-38)	0.62

Demographics for all study subjects (n=214) with acute rhinovirus infection vs. control (non-detectable virus). *IQR*, interquartile range. P-values based on Wilcoxon rank-sum test for continuous variables; χ^2 test for categorical.

Accepted Article

This article is protected by copyright. All rights reserved.

TABLE 2 .Baseline characteristics for severely premature subjects

	Control n=17	Rhinovirus n=28	p-value
Male, n (%)	14(82)	18(64)	0.16
*Age (years), median (IQR)	0.78 (0.34-0.86)	1.02 (0.72-1.41)	0.08
Black, n (%)	10(59)	21(78)	0.26
Gestational age (weeks), median (IQR)	25 (25-27)	26 (25-27)	0.98
Family history of asthma (y/n), n (%)	2(11)	9(32)	0.08
Bronchopulmonary dysplasia (y/n), n (%)	10(58)	20(71)	0.39
NICU mechanical ventilation (y/n), n (%)	12(76)	22(78)	0.87
Necrotizing enterocolitis (y/n), n (%)	4(23)	6(21)	0.87
Intraventricular hemorrhage (y/n), n (%)	6(35)	5(17)	0.20

Demographic and clinical characteristics of premature subjects with acute rhinovirus infection vs. control (non-detectable virus) included in electronic medical record (EMR) review (n=45). *IQR*, interquartile range; *NICU*, neonatal intensive care unit.
*Age at the time of testing
P-values based on Wilcoxon rank-sum test for continuous variables; χ^2 test for categorical.

This article is protected by copyright. All rights reserved.

Accepted Article

TABLE 3. Multivariate predictive models for rhinovirus-induced airway IL-4 and IL-17 in severely premature children

	IL-4 pg/ml		IL-17 pg/ml	
Characteristics	Full model [a]	p-value	Full model [a]	p-value
BPD	11.97 ± 4.50	0.02	6.90± 3.01	0.04
NEC	3.53 ± 5.10	0.50	6.40 ± 3.41	0.08
IVH	2.89 ± 2.11	0.19	2.42 ± 1.41	0.10
Family history of asthma	-1.50 ± 3.53	0.67	0.37± 2.36	0.87
NICU mechanical ventilation	-0.941 ± 4.21	0.82	0.18± 2.81	0.94

[a] Adjusted for age, gender and race. Data presented as β-coefficient ± SE and p-value obtained by multiple linear regression.
BPD, bronchopulmonary dysplasia; *NEC*, necrotizing enterocolitis; *IVH*, Intraventricular hemorrhage; *NICU*, neonatal intensive care unit.

TABLE 4. PICU admission and rhinovirus-induced airway IL-4 and IL-17 in severely premature children

	Crude OR		Adjusted OR[b]	
Predictors of any PICU admission (y/n)[a]	OR (95%CI)	p-value	OR (95%CI)	p-value
RV-induced high IL-4	8.25 (1.53-44.5)	0.01	8.72 (1.3-58.7)	0.02
RV-induced high IL-17	4.18 (1.13-15.4)	0.03	5.22 (1.1-24.5)	0.03
BPD	1.83 (0.5-6.9)	0.37	1.65 (0.5-7.6)	0.53

[a] History of any admission to pediatric intensive care unit (PICU) in the first two years of life.
[b] Adjusted for age, gender and race. P-value obtained by logistic regression.
RV, rhinovirus; *BPD*, bronchopulmonary dysplasia

This article is protected by copyright. All rights reserved.

TABLE 5. Respiratory hospitalizations and rhinovirus-induced IL-4 and IL-17 in severely premature children

RV-induced high IL-4 (y/n)	Yes (n=11)	No (n=34)	p-value
Respiratory hospitalizations (hosp/year)[a] , median (IQR) [b]	3.65 (95% CI 2.8-4.8)	0.32 (95% CI 0-3.4)	<0.01
RV-induced high IL-17 (y/n)	**Yes (n=16)**	**No (n=28)**	
Respiratory hospitalizations (hosp/year)[a] , median (IQR) [b]	3.0 (95% CI 1.1-3.9)	0.7 (95% CI 0-3.4)	0.11

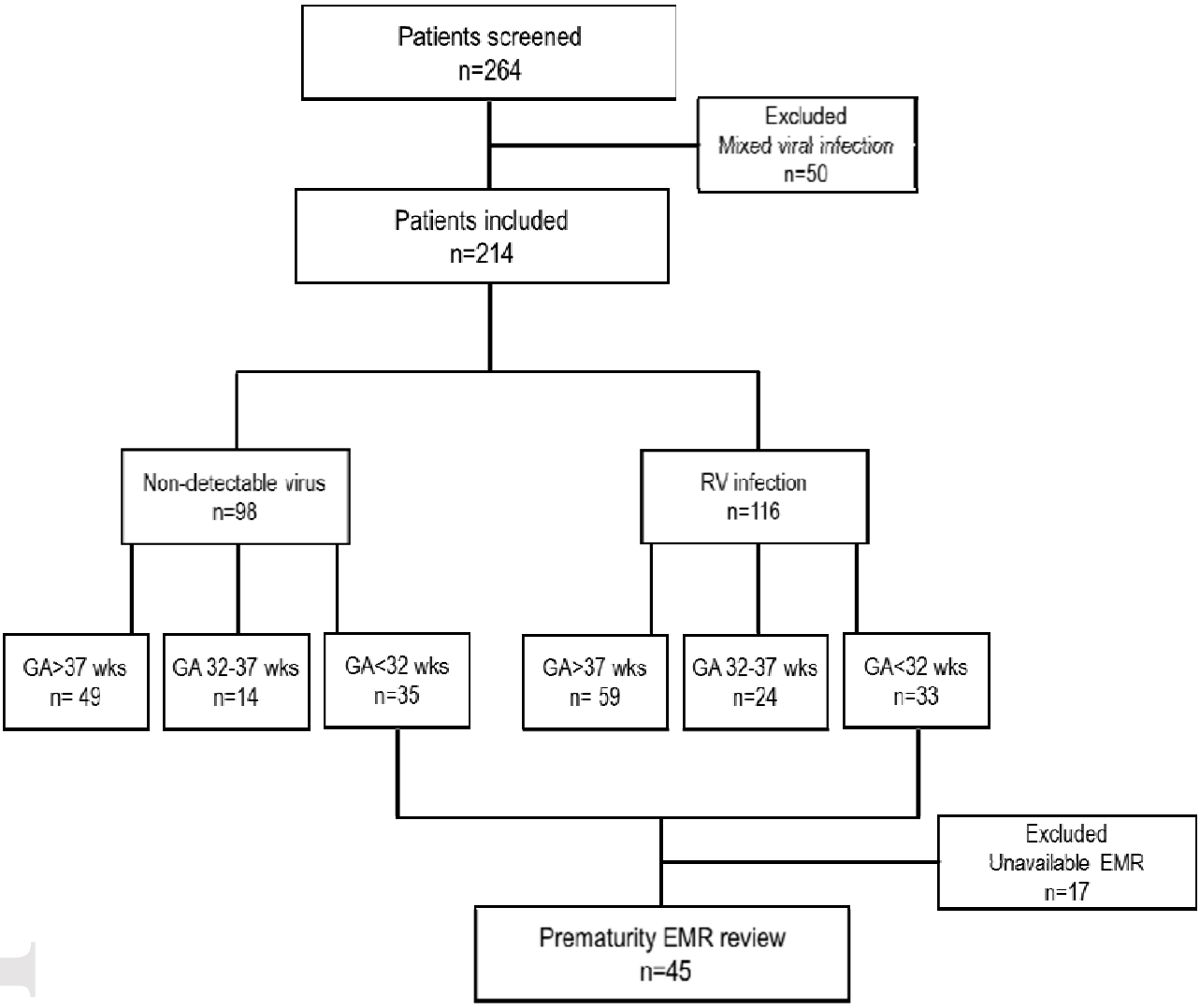
Predictors of respiratory hospitalizations (hosp/year)[a]	Full model [c]	p-value
RV-induced high IL-4	2.51 ± 0.73	<0.01
Family history of asthma	2.90 ± 0.88	<0.01
RV-induced high IL-17	1.42 ± 0.70	0.05
BPD	0.82 ± 0.83	0.32
NICU mechanical ventilation	0.73 ± 0.98	0.46

[a] Number of respiratory admissions per year during the first two years of life.
[b] *IQR*, interquartile; p-value obtained by Wilcoxon rank-sum test
[c] Adjusted for gender and race. Data presented as β-coefficient ± SE; p-value obtained by multiple linear regression.
RV, rhinovirus; *BPD*, bronchopulmonary dysplasia; *NICU*, neonatal intensive care unit.

This article is protected by copyright. All rights reserved.

Accepted Article

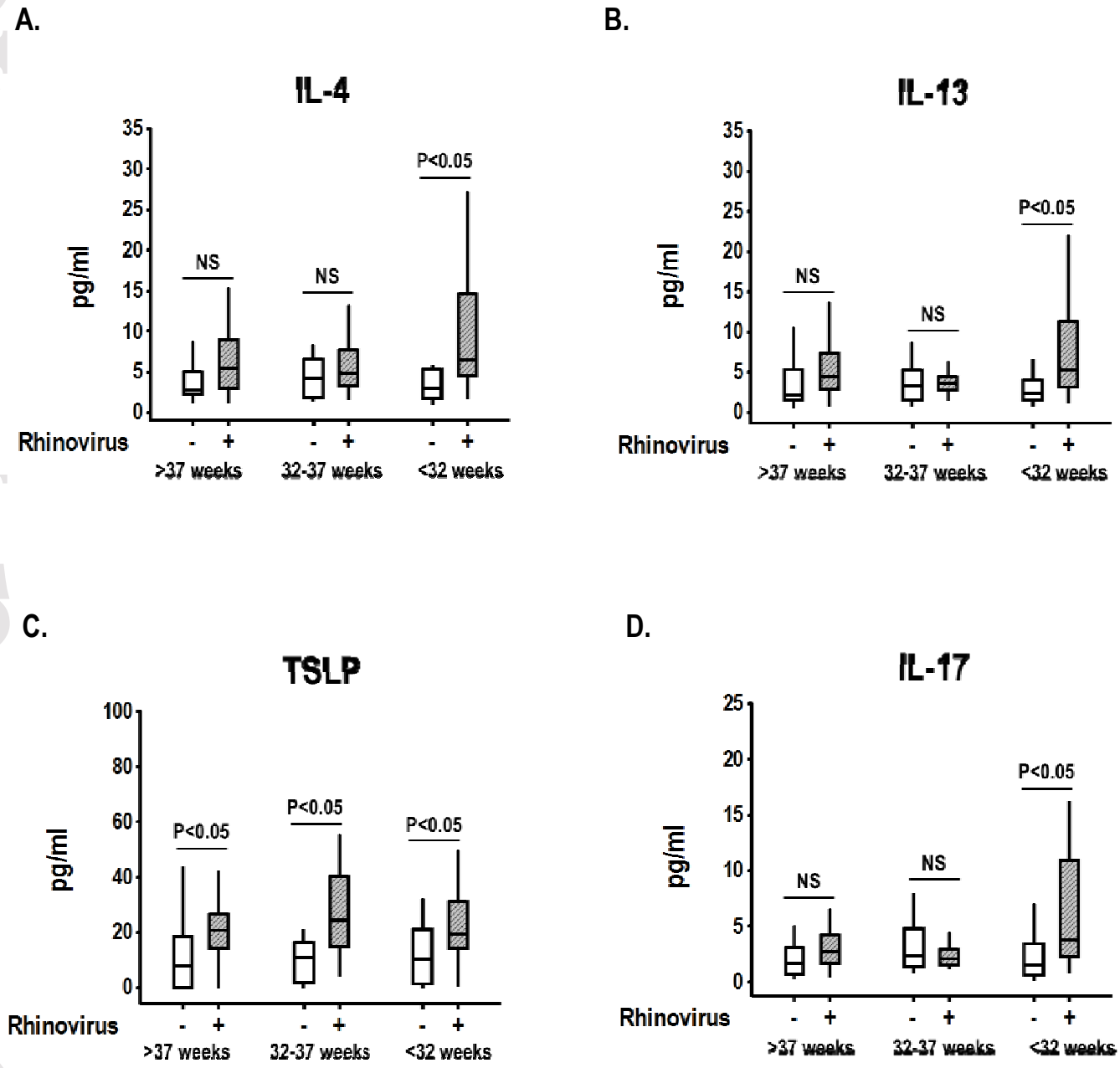
FIGURE 1.



Study design flow chart. *RV*, rhinovirus; *GA*, gestational age; *EMR*, electronic medical records

This article is protected by copyright. All rights reserved.

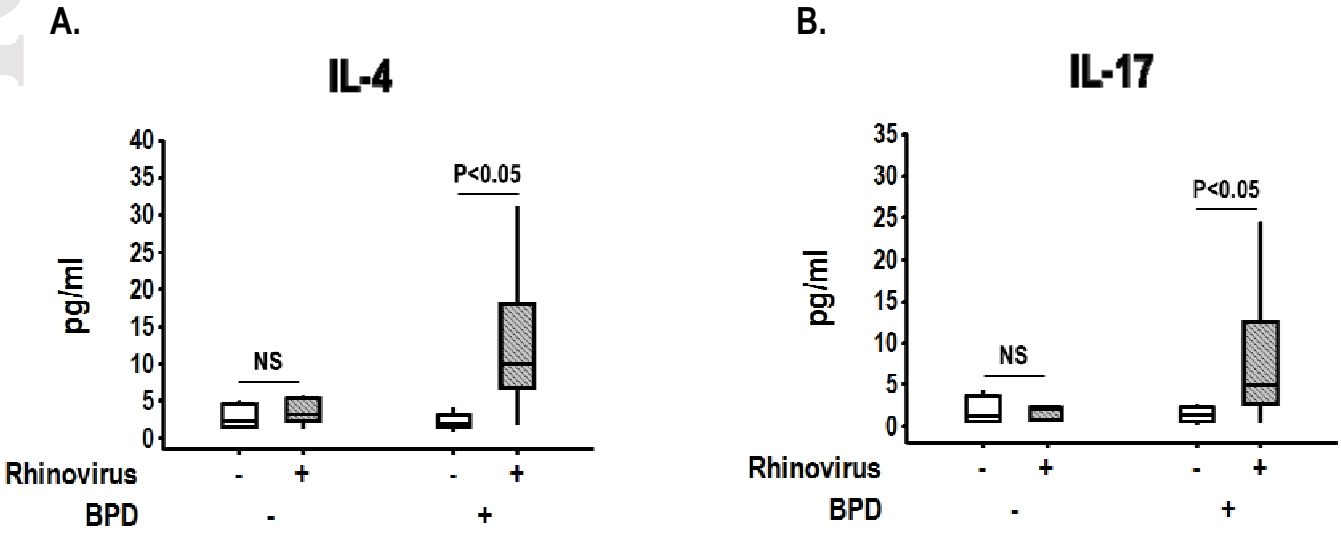
FIGURE 2.



Rhinovirus-induced Th2 and Th17 nasal cytokines in premature and full-term children. Left boxplots represent full-term subjects (>37 wks) with RV (n=59) vs. control (n=49). Middle boxplots are preterm subjects (32-37 wks) with RV (n=24) vs. control (n=14). Right boxplots represent severely premature subjects (<32 wks) with RV (n=33) vs. control (n=35).

This article is protected by copyright. All rights reserved.

FIGURE 3.



Effect of bronchopulmonary dysplasia (BPD) in rhinovirus-induced Th2 and Th17 nasal airway cytokines in severely premature children. Left boxplots represent severely premature subjects without BPD (RV-infected n=8 vs. uninfected n=7). Right boxplots represent severely premature subjects with BPD (RV-infected n=20 vs. uninfected n=10).

This article is protected by copyright. All rights reserved.