

Association of type 2 cytokines in severe rhinovirus bronchiolitis during infancy with risk of developing asthma: A multicenter prospective study

To the Editor,

Bronchiolitis is the leading cause of hospitalizations in U.S. infants.¹ In addition to the acute morbidity, cohort studies have also shown that 30%–40% of infants hospitalized for bronchiolitis (severe bronchiolitis) develop childhood asthma.¹ Particularly, early life infection with rhinovirus (RV)—the second most common pathogen of bronchiolitis—is associated with an increased risk of childhood asthma.¹ Yet, the mechanism through which RV raises asthma risk in infants is largely unknown. Experimental models and human (cross-sectional and retrospective) studies have reported that RV infection may induce type 2 cytokines (eg, interleukin [IL]-4, IL-5, IL-13, thymic stromal lymphopoietin [TSLP]) and that the levels of these cytokines are elevated in the asthmatic airway.^{2–4} However, no prospective study has investigated the longitudinal relation of type 2 airway inflammation in children—let alone infants with bronchiolitis—to the development of childhood asthma. To address this knowledge gap, we prospectively examined the association of nasopharyngeal cytokines in infants with RV bronchiolitis with the risk of developing childhood asthma, by using data from a multicenter cohort of infants with severe bronchiolitis.

Details of the study design, samples, measurement, and analysis may be found in the Methods S1). Briefly, this multicenter prospective cohort study, the 35th Multicenter Airway Research Collaboration (MARC-35),^{5,6} enrolled 1016 infants (aged < 12 months) hospitalized for bronchiolitis at 17 sites across 14 US states (Table S1) during the 2011–2014 winter seasons. Bronchiolitis was defined by the American Academy of Pediatrics guidelines.⁷ In addition to the phenotypic data measurement via structured interview and medical record review, nasopharyngeal airway samples were collected within 24 hours of hospitalization using a standardized protocol.^{5,6} Levels of 10 nasopharyngeal cytokines (IL-4, IL-5, IL-10, IL-12, IL-13, IL-25, IL-33, interferon [IFN]- β , macrophage inflammatory protein [MIP]-1 α , and TSLP) were quantified using electrochemiluminescence immunoassays. Respiratory viruses were tested using real-time PCR assays for RV and respiratory syncytial virus (RSV). To quantify the relative RV genomic load, cycle threshold (CT) values—the number of amplification cycles needed for a positive PCR test result—were used. The primary outcome was asthma at age 4 years based on a commonly used epidemiologic definition—that is, physician diagnosis of asthma plus either asthma medication use or asthma-related symptoms in the past year.⁸ For the current study, we analyzed 132 infants with RV bronchiolitis who underwent nasopharyngeal

cytokine measurement. To examine the association of exposures (cytokine levels and RV genomic load) with asthma, we used generalized linear mixed-effects models, adjusting for potential confounders (age, sex, parental history of asthma, breathing problems prior to enrollment, IgE sensitization [aeroallergens or food], and virology [solo RV, RV/RSV coinfection]) and hospital-level clustering. As the models indicated statistically significant virology-exposure interactions, we then stratified the analysis by virology.

Of 132 infants with severe RV bronchiolitis, the median age was 4 (IQR 2–6) months, 64% were male, 40% were non-Hispanic white, and 55% had RSV coinfection. Asthma was observed in 30% of children at age 4 years (Tables 1 and S2). There were statistically significant interactions between virology and six cytokine levels (IL-4, IL-5, IL-13, IFN- β , MIP-1 α , and TSLP) on the asthma risk (P-interaction < 0.05), indicating that exposure-asthma associations differ between solo RV infection and RV/RSV coinfection. Indeed, in infants with solo RV infection, the cytokine levels (IL-4, IL-5, and TSLP) and RV genomic load significantly differed between those with and without asthma (unadjusted P < 0.05; Table S3) while there were no differences in those with RV/RSV (unadjusted P > 0.05). Heatmap (Figure S1) also showed that distributions of these type 2 cytokines differed by virology and outcome. In the adjusted analysis (Figures 1 and S2), only infants with solo RV bronchiolitis had significant associations of higher type 2 cytokine levels (IL-4, IL-5, IL-13, and TSLP) with an increased risk of asthma (adjusted P < 0.05). In the sensitivity analysis using normalized cytokine levels and the subgroup analysis excluding infants with a breathing problem prior to enrollment, the results were similar (Figures S3 and S4).

These findings are concordant with previous cross-sectional and retrospective studies that suggested potential interrelations between RV infection, type 2 cytokines (eg, IL-4, IL-5, IL-13, TSLP), and asthmatic airway inflammation.^{2–4} The current prospective study builds on these earlier reports and extends them by demonstrating the prospective association between type 2 cytokine levels in the airway of infants with solo RV bronchiolitis and the risk of developing asthma. The mechanisms underlying these findings warrant further investigation. It is possible that severe RV infection is an early marker of T_H2 bias in predisposed infants, which leads to augmented RV infection and replication.^{3,4} Yet, our data also showed no significant correlations between serum total IgE levels and nasopharyngeal cytokine levels (Table S4). Alternatively, the association may be

TABLE 1 Characteristics and clinical presentation of 132 infants hospitalized for rhinovirus bronchiolitis, by asthma status at age 4 y

Variables	No asthma n = 92 (70%)	Asthma n = 40 (30%)	P-value
Characteristics at hospitalization for bronchiolitis			
Age (mo), median, (IQR)	4 (2-6)	5 (3-9)	0.02
Male sex	55 (60)	29 (72)	0.23
Race/ethnicity			
Non-Hispanic white	42 (46)	11 (28)	0.21
Non-Hispanic black	20 (22)	14 (35)	
Hispanic	27 (29)	14 (35)	
Other	3 (3)	1 (3)	
Parental history of asthma	29 (32)	22 (55)	0.02
Parental history of eczema	14 (15)	13 (33)	0.04
Maternal smoking during pregnancy	11 (12)	4 (10)	0.60
Mode of birth (c-section)	24 (26)	14 (35)	0.40
Prematurity (32-37 wk)	19 (21)	10 (25)	0.75
Previous breathing problems before the index hospitalization ^a	20 (22)	17 (43)	0.03
History of eczema	10 (11)	11 (28)	0.03
Ever attended daycare	28 (30)	13 (33)	0.98
Household sibling	74 (80)	35 (88)	0.46
Breastfed	42 (46)	21 (53)	0.64
Smoke exposure at home	13 (14)	2 (5)	0.22
Corticosteroid use in lifetime	11 (12)	16 (40)	0.001
Presentation and course at hospitalization for bronchiolitis			
Weight at presentation (kg), median (IQR)	6.2 (4.9-7.7)	7.4 (5.7-8.8)	0.02
Laboratory testing			
Coinfection with RSV	58 (63)	15 (38)	0.01
Blood eosinophil count/ μ L, median (IQR)	170 (70-30)	100 (20-200)	0.16
Serum total 25OHD (ng/mL), median (IQR)	24.8 (16.9-31.2)	26.7 (18.5-34.0)	0.51
Serum LL-37 (ng/mL), median (IQR)	54.0 (40-70)	62.0 (42.0-75.0)	0.29
sIgE sensitization ^b	9 (10)	10 (25)	0.04
Food sensitization	9 (10)	10 (25)	0.04
Aeroallergen sensitization	1 (1)	0 (0)	0.99
Clinical course			
Intensive care use ^c	12 (13)	5 (13)	0.99
Use of mechanical ventilation	2 (2)	2 (5)	0.75
Chronic outcome			
Recurrent wheeze by age 3 y ^d	27 (29)	31 (78)	<0.001

Data are no. (%) of infants unless otherwise indicated. Percentages may not equal 100, because of rounding and missingness.

25OHD, 25-hydroxyvitamin D; IQR, interquartile range; RSV, respiratory syncytial virus; sIgE, specific immunoglobulin E.

^aDefined as an infant having cough that wakes him/her at night and/or causes emesis, or when the infant has wheezing or shortness of breath without cough before the index hospitalization.

^bDefined by having one or more positive values for serum allergen-specific IgE at the index hospitalization.

^cDefined as admission to intensive care unit and/or use of mechanical ventilation (continuous positive airway pressure and/or intubation during inpatient stay, regardless of location) at any time during the index hospitalization.

^dDefined as having at least two corticosteroid-requiring exacerbations in 6 mo or having at least four wheezing episodes in 1 y that last at least 1 d and affect sleep.

causal—that is, severe RV bronchiolitis modulates host airway response toward type 2 inflammation and damages the airways during early infancy—a crucial period of lung development.¹ Furthermore, these possibilities are not mutually exclusive and may jointly

contribute to disrupted epithelial barrier, exaggerated inflammation, and airway injury, thereby leading to the development of asthma. Notwithstanding the complexity, our data, in conjunction with earlier studies, provide an evidence base for early identification of children

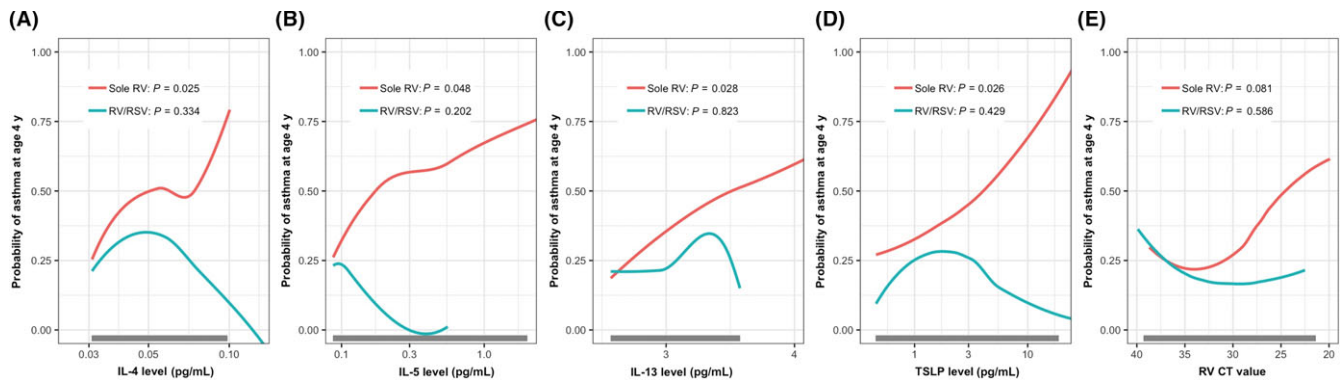


FIGURE 1 The association of nasopharyngeal type 2 cytokine levels and rhinovirus genomic load in infants hospitalized for bronchiolitis with risks of asthma at age 4 y, according to virology. The fitted lines represent locally estimated scatterplot smoothed (loess) curves for infants with solo RV infection and those with RV/RSV coinfection. Only in infants with solo bronchiolitis, there were significant associations of nasopharyngeal (A) IL-4, (B) IL-5, (C) IL-13, (D) TSLP levels, and (E) RV genomic load (lower cycle threshold [CT] value indicates higher genomic loads) with a significantly higher risk of asthma at age 4 y with the use of generalized linear mixed-effects model. The gray bar represents the range in which 95% of data are present. The results of all cytokines are presented in Figures S2 and S3. IL, interleukin; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin

at high risk for asthma, as well as the development of targeted prevention strategies (eg, immunomodulators).

Our study has potential limitations. First, bronchiolitis involves inflammation of the lower airways in addition to the upper airways. Although our study was based on the nasopharyngeal samples, studies have shown that upper airway sampling provides reliable representation of the lung inflammatory profiles.⁹ Second, the study did not have information from “healthy controls.” However, the objective was to examine the role of RV infection and host response on the development of asthma among infants with severe bronchiolitis. Third, we used an epidemiologic definition of asthma at age 4 years while diagnosis of asthma at this age can be challenging. To address this important point, the study population is currently being followed longitudinally up to age 6 years. Finally, although MARC-35 samples consist of racially/ethnically and geographically diverse US sample, our findings may not be generalizable beyond infants with severe RV bronchiolitis (eg, infants with solo RSV infection). Regardless, bronchiolitis accounts for 130 000 hospitalizations annually, with RV being the second most common causative virus.¹ Additionally, further understanding of infants at highest risk could better delineate the mechanisms of asthma development in larger populations.

In summary, higher type 2 cytokine (IL-4, IL-5, IL-13, and TSLP) levels in the airway of infants with solo RV infection were associated with a greater risk of developing childhood asthma. Our data should advance research not only into understanding the mechanisms linking early RV infection to incident asthma but also into developing potential strategies for asthma prevention.

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CONFLICTS OF INTEREST

Dr. Hasegawa received asthma-related research grants from Novartis and Teva, unrelated to the current work. Dr. Celedón received research materials from GSK and Merck (inhaled steroids) and Pharmavite (vitamin D and placebo capsules) to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work. The other authors have no financial relationships relevant to this article to disclose.

Keywords

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

ONLINE SUPPLEMENT

Association of type 2 cytokines in severe rhinovirus bronchiolitis during infancy with risk of developing asthma: A multicenter prospective study

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Figure E2. Association of nasopharyngeal cytokine levels in infants hospitalized for bronchiolitis with risk of asthma at age 4 years, according to virology

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Figure E5 Correlations between nasopharyngeal airway interleukin-5 levels and blood eosinophil count in infants hospitalized with bronchiolitis

SUPPLEMENTAL METHODS

Study Design, Setting, and Participants

This is a pre-planned analysis of data from a multicenter prospective cohort study of infants hospitalized with bronchiolitis (severe bronchiolitis). This study, called the 35th Multicenter Airway Research Collaboration (MARC-35),^{1,2} was coordinated by the Emergency Medicine Network (EMNet), an international research collaboration with 245 participating hospitals.³

Using a standardized protocol, investigators at 17 sites across 14 U.S. states (**Table E1**) enrolled infants (age <1 year) hospitalized with an attending physician diagnosis of bronchiolitis during three consecutive bronchiolitis seasons (from November 1 to April 30) during 2011-2014. Bronchiolitis was defined by the American Academy of Pediatrics guidelines – acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions.⁴ We excluded infants who were transferred to a participating hospital >24 hours after the original hospitalization, those who were consented >24 hours after hospitalization, or those with known heart-lung disease, immunodeficiency, immunosuppression, or gestational age <32 weeks. All patients were treated at the discretion of the treating physician. The institutional review board at each of the participating hospitals approved the study. Written informed consent was obtained from the parent or guardian.

Data Collection

Investigators conducted a structured interview that measured patients' demographic characteristics, medical, family, and environmental history, and details of the acute illness. Emergency department and hospital medical record reviews provided further clinical data, such

as vital signs, physical examination, medical management, and disposition. After the index hospitalization for bronchiolitis, interviewers began interviewing parents/legal guardians by telephone at 6-month intervals, in addition to medical record review by trained physicians. All data were reviewed at the EMNet Coordinating Center at Massachusetts General Hospital (Boston, MA), and site investigators were queried about missing data and discrepancies identified by manual data checks.

Nasopharyngeal Airway Sampling

In addition to the clinical data, investigators also collected nasopharyngeal airway samples within 24 hours of hospitalization by using a standardized protocol that was utilized in a previous cohort study of children with bronchiolitis.^{5,6} All of the sites used the same collection equipment (Medline Industries, Mundelein, IL) and collected the samples within 24 hours of a child's arrival on the medical ward or intensive care unit. For the collection, the infant was placed supine, 1 mL of normal saline was instilled into one naris, and then an 8 French suction catheter was used to remove the mucus. This procedure was performed once on each nostril. After the sample collection from both nares, 2 mL of normal saline was suctioned through the catheter to clear the tubing and to ensure that a standard volume of aspirate is obtained. These samples were tested for respiratory viruses and cytokines.

Respiratory Viruses and Genomic Load Measurement

We identified respiratory pathogens by using singleplex or duplex two-step real-time PCR (rtPCR) at Baylor College of Medicine (Houston, TX, USA). We used real-time reverse transcriptase-PCR to detect RNA respiratory viruses, including RV,⁷ respiratory syncytial virus

[RSV],^{8,9} and 12 other RNA viruses. In addition, we also quantified relative RV genomic load as the number of amplification cycles needed for a positive PCR test result (cycle threshold [CT]).^{5,10} Studies showed that CT values provide a semi-quantitative measure of genomic load, with a significant inverse relationship between genomic load and CT values.^{11,12} All rtPCR assays were tested in duplicate and samples with incongruent values (one well positive) were retested. To reduce carryover contamination, sample preparation, RNA/DNA extraction, cDNA and amplification were performed in separate areas. All PCR runs had extraction and reagent positive and negative controls.

Nasopharyngeal Cytokine Measurement

We also measured the levels of 10 cytokines (interleukin [IL]-4, IL-5, IL-10, IL-12, IL-13, IL-25, IL-33, interferon [IFN]- β , macrophage inflammatory protein[MIP]-1 α , and thymic stromal lymphopoietin [TSLP]) in the nasopharyngeal samples of infants with bronchiolitis, using multiplex the Meso Scale Discovery (MSD) electrochemiluminescent R-Plex multiplex immunoassay (Meso Scale Diagnostics, Rockville, MD), on the MESO QuickPlex SQ 120 system (Meso Scale Diagnostics). Discovery Workbench 4.0 software (Meso Scale Diagnostics) was used to analyze the data, including quality control. All samples were run in duplicate. The cytokine concentrations that were calculated to be less than or equal to the detection limit of the assay were considered equal to the detection limit for analysis purposes.

Outcome Measure

The outcome measure of interest was asthma at age 4 years based on a commonly-used epidemiologic definition of asthma¹³ – i.e., physician diagnosis of asthma, plus either asthma

medication use (e.g., albuterol inhaler, inhaled corticosteroids, montelukast) or asthma-related symptoms (e.g., wheezing, nocturnal cough, chest tightness) in the past year.

Statistical Analyses

For the current study, of 187 infants with RV detection in the cohort, we analyzed 132 infants who underwent nasopharyngeal cytokine measurement. These infants had sufficient amount of nasopharyngeal airway sample for the cytokine testing. There were no significant differences in patient characteristics or clinical presentation between the analytic and non-analytic cohorts (all $P > 0.05$; **Table E2**). First, we compared the patient characteristics, clinical presentation, virology, and laboratory data at enrollment by asthma status at age 4 years, with the use of chi-square test or Wilcoxon rank sum test as appropriate.

Next, to examine the association of exposures (log-transformed cytokine levels and RV genomic load) at enrollment with asthma at age 4 years, we constructed generalized linear mixed models, adjusting for potential confounders (age, sex, parental history of asthma, previous breathing problems prior to enrollment, IgE sensitization [aeroallergens or food], and virology [solo RV, RV/RSV coinfection] at enrollment) and patient clustering at the hospital-level. As the models indicated statistically significant virology–exposure interactions ($P < 0.05$ with likelihood ratio tests), we then stratified the analysis by virology (solo RV, RV/RSV coinfection) with adjusting for age, sex, and patient clustering. In the sensitivity analyses, to address potential variable dilution of the nasopharyngeal aspirate specimens, we normalized the cytokine levels using RNase P (host housekeeping gene) copy number, as previously described.^{2,14} We also repeated the analysis excluding 37 infants who had a breathing problem prior to the enrollment.

Analyses used R version 3.5. All P-values were two-tailed, with $P < 0.05$ considered statistically significant.

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Table E1. Principal investigators at the 17 participating sites in MARC-35

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Michelle B. Dunn, MD and Jonathan M. Spergel, MD, PhD	Children's Hospital of Philadelphia, Philadelphia, PA
Juan C. Celedón, MD, DrPH	Children's Hospital of Pittsburgh, Pittsburgh, PA
Michael R. Gomez, MD, MS-HCA and Nancy Inhofe, MD	The Children's Hospital at St. Francis, Tulsa, OK
Brian M. Pate, MD and Henry T. Puls, MD	The Children's Mercy Hospital & Clinics, Kansas City, MO
Stephen J. Teach, MD, MPH	Children's National Medical Center, Washington, D.C.
Richard T. Strait, MD and Stephen C. Porter, MD, MSc, MPH	Cincinnati Children's Hospital and Medical Center, Cincinnati, OH
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Michelle D. Stevenson, MD, MS	Norton Children's Hospital, Louisville, KY
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Charles G. Macias, MD, MPH	Texas Children's Hospital, Houston, TX

Table E2. Characteristics and clinical presentation of analytic and non-analytic cohorts

Variables	Analytic cohort n=132 (71%)	Non-analytic cohort n=55 (29%)	P-value
Characteristics			
Age (mo), median, (IQR)	3.9 (2.0-6.4)	3.0 (2.0-6.5)	0.67
Female sex	48 (36.4)	14 (25.5)	0.27
Race/ethnicity			0.90
Non-Hispanic white	52 (39.4)	19 (34.5)	
Non-Hispanic black	34 (25.8)	14 (25.5)	
Hispanic	41 (31.1)	20 (36.4)	
Other	4 (3.0)	2 (3.6)	
Parental history of asthma	51 (38.6)	18 (32.7)	0.59
Parental history of eczema	27 (20.5)	8 (14.5)	0.51
Maternal smoking during pregnancy	15 (11.4)	7 (12.7)	0.52
Mode of birth (c-section)	38 (28.8)	17 (30.9)	0.52
Prematurity (32-37 weeks)	29 (22.0)	12 (21.8)	0.81
Previous breathing problems before the index hospitalization*	0.34 (0.58)	0.38 (0.68)	0.64
History of eczema	21 (15.9)	4 (7.3)	0.23
Ever attended daycare	41 (31.1)	16 (29.1)	0.78
Household sibling	109 (82.6)	42 (76.4)	0.45
Breastfed	62 (47.0)	17 (30.9)	0.06
Smoke exposure at home	15 (11.4)	4 (7.3)	0.56
Corticosteroid use in lifetime	27 (20.5)	10 (18.2)	0.76
Presentation and course at hospitalization for bronchiolitis			
Weight at presentation (kg), median (IQR)	6.7 (5.2-7.9)	6.1 (4.9-8.0)	0.52
Laboratory testing			
Coinfection with RSV	72 (54.5)	37 (67.3)	0.24
Blood eosinophil count/ul, median (IQR)	130 (40-270)	160 (70-240)	0.84
Serum total 25OHD (ng/ml), median (IQR)	25.0 (16.9-33.0)	28.6 (20.8-35.2)	0.09
Serum LL-37 (ng/ml), median (IQR)	54.0 (40.0-71.0)	54.0 (35.0-67.0)	0.05
sIgE sensitization†	19 (14.4)	14 (25.5)	0.16
Food sensitization	19 (14.4)	14 (25.5)	0.16
Aeroallergen sensitization	1 (0.8)	0 (0.0)	0.66
Clinical course			
Intensive care use‡	17 (12.9)	11 (20.0)	0.38
Use of mechanical ventilation	4 (3.0)	3 (5.5)	0.60

Data are no. (%) of children unless otherwise indicated. Percentages may not equal 100, because of rounding and missingness

Abbreviations: IQR, interquartile range; RSV, respiratory syncytial virus; sIgE, specific immunoglobulin E; 25OHD, 25-hydroxyvitamin D

* Defined as an infant having cough that wakes him/her at night and/or causes emesis, or when the child has wheezing or shortness of breath without cough before the index hospitalization

† Defined by having one or more positive values for serum allergen-specific IgE at the index hospitalization

‡ Defined as admission to intensive care unit and/or use of mechanical ventilation (continuous positive airway pressure and/or intubation during inpatient stay, regardless of location) at any time during the index hospitalization

Table E3. Nasopharyngeal cytokine levels and rhinovirus genomic load in infants hospitalized for rhinovirus bronchiolitis by asthma status according to the virology

Group and exposure	No asthma	Asthma	P-value*
Overall (n=132) †			
IL-4 (pg/ml)	0.05 [0.03, 0.08]	0.06 [0.03, 0.08]	0.48
IL-5 (pg/ml)	0.10 [0.09, 0.11]	0.11 [0.10, 0.18]	0.03
IL10 (pg/ml)	3.86 [0.89, 11.42]	3.61 [1.41, 7.06]	0.83
IL-12 (pg/ml)	0.24 [0.19, 0.26]	0.22 [0.19, 0.26]	0.75
IL-13 (pg/ml)	3.47 [2.88, 3.54]	3.47 [2.96, 3.54]	0.23
IL-25 (pg/ml)	0.38 [0.36, 0.59]	0.38 [0.36, 1.22]	0.24
IL-33 (pg/ml)	3.29 [1.11, 12.97]	4.61 [1.72, 16.16]	0.32
IFN- β (pg/ml)	19.83 [5.62, 44.31]	9.25 [5.76, 30.24]	0.36
MIP-1 α (pg/ml)	160.68 [65.95, 326.14]	194.47 [71.65, 396.29]	0.63
TSLP (pg/ml)	1.32 [0.73, 3.01]	2.00 [0.95, 3.54]	0.13
RV CT value	30 [27, 35]	28 [24, 33]	0.03
Solo RV infection (n=59)			
IL-4 (pg/ml)	0.03 [0.03, 0.08]	0.08 [0.04, 0.08]	0.04
IL-5 (pg/ml)	0.10 [0.10, 0.11]	0.11 [0.11, 0.57]	0.01
IL10 (pg/ml)	2.71 [0.53, 7.53]	4.54 [1.48, 7.45]	0.51
IL-12 (pg/ml)	0.19 [0.17, 0.26]	0.19 [0.19, 0.26]	0.85
IL-13 (pg/ml)	3.21 [2.96, 3.54]	3.47 [3.47, 3.54]	0.10
IL-25 (pg/ml)	0.38 [0.36, 0.48]	0.38 [0.36, 1.22]	0.34
IL-33 (pg/ml)	5.56 [1.24, 14.46]	4.00 [1.73, 15.97]	0.79
IFN- β (pg/ml)	5.76 [2.82, 12.74]	7.27 [5.76, 35.47]	0.10
MIP-1 α (pg/ml)	173.44 [73.20, 315.42]	266.21 [166.53, 430.22]	0.12
TSLP (pg/ml)	1.27 [0.73, 2.55]	2.83 [0.97, 5.47]	0.03
RV CT value	27 [25, 31]	24 [23, 27]	0.046
RV/RSV coinfection (n=73)			
IL-4 (pg/ml)	0.06 [0.03, 0.08]	0.04 [0.03, 0.08]	0.36
IL-5 (pg/ml)	0.10 [0.09, 0.11]	0.10 [0.09, 0.10]	0.38
IL10 (pg/ml)	4.64 [1.30, 11.49]	2.92 [1.39, 4.14]	0.41
IL-12 (pg/ml)	0.26 [0.19, 0.26]	0.26 [0.19, 0.26]	0.83
IL-13 (pg/ml)	3.47 [2.73, 3.54]	3.47 [2.80, 3.47]	0.70
IL-25 (pg/ml)	0.37 [0.30, 1.07]	0.38 [0.36, 1.22]	0.58
IL-33 (pg/ml)	2.72 [1.12, 10.64]	4.74 [1.88, 14.99]	0.32
IFN- β (pg/ml)	28.57 [13.78, 48.58]	14.21 [7.85, 26.69]	0.08
MIP-1 α (pg/ml)	152.37 [65.44, 404.15]	73.04 [28.89, 229.68]	0.17
TSLP (pg/ml)	1.45 [0.75, 3.49]	1.35 [0.98, 2.01]	0.77
RV CT value	32 [28, 36]	35 [29, 37]	0.35

Data are presented as median [IQR]. Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

* Wilcoxon rank sum test. Bold results indicate P<0.05

† Correlations of cytokine levels with total IgE, RV CT value, and blood eosinophil count are shown in **Table E4**, **Table E5**, and **Figure E5**, respectively.

Table E4. Correlations between nasopharyngeal cytokine levels and serum total IgE levels in infants hospitalized for rhinovirus bronchiolitis, according to the virology

Cytokines	Overall		Solo RV infection		RV/RSV coinfection	
	Spearman's rho	P-value	Spearman's rho	P-value	Spearman's rho	P-value
IL-4	-0.02	0.86	-0.07	0.62	0.00	0.86
IL-5	0.17	0.06	0.21	0.11	0.02	0.64
IL10	-0.14	0.11	-0.08	0.56	0.06	0.10
IL-12	0.03	0.74	-0.02	0.90	-0.20	0.27
IL-13	-0.04	0.67	-0.09	0.49	0.13	0.81
IL-25	0.06	0.50	0.05	0.70	-0.03	0.58
IL-33	0.06	0.47	-0.01	0.91	0.07	0.33
IFN- β	0.00	0.98	0.18	0.17	0.12	0.52
MIP-1 α	-0.06	0.50	0.03	0.79	-0.08	0.08
TSLP	0.08	0.37	0.14	0.29	-0.21	0.92

Abbreviations: IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

Table E5. Correlations between rhinovirus cycle threshold value and nasopharyngeal cytokine levels in infants hospitalized for rhinovirus bronchiolitis, according to the virology

Cytokines	Overall		Solo RV infection		RV/RSV coinfection	
	Spearman's rho	P-value	Spearman's rho	P-value	Spearman's rho	P-value
IL-4	-0.06	0.51	-0.23	0.09	1.00	0.80
IL-5	-0.24	0.01	-0.45	<0.001	0.03	0.40
IL10	0.06	0.53	-0.20	0.13	0.10	0.26
IL-12	0.07	0.46	0.09	0.51	0.13	0.87
IL-13	-0.08	0.34	-0.19	0.15	-0.02	0.48
IL-25	-0.07	0.46	0.03	0.82	0.09	0.44
IL-33	0.04	0.64	0.11	0.41	-0.09	0.54
IFN- β	0.19	0.03	-0.18	0.16	0.07	0.80
MIP-1 α	-0.08	0.37	-0.11	0.39	0.03	0.77
TSLP	-0.02	0.81	-0.23	0.08	0.03	0.33

Abbreviations: IFN, interferon; IL, interleukin; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

Figure E1. Relations of nasopharyngeal cytokine levels and rhinovirus genomic load in infants hospitalized for bronchiolitis with virology and asthma outcome at age 4 years.

The heatmap shows the distributions of nasopharyngeal cytokine levels and RV genomic load by virology and asthma status at age 4 years, with the use of hierarchical clustering with average linkage algorithm.

Abbreviations: IFN, interferon; IL, interleukin; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

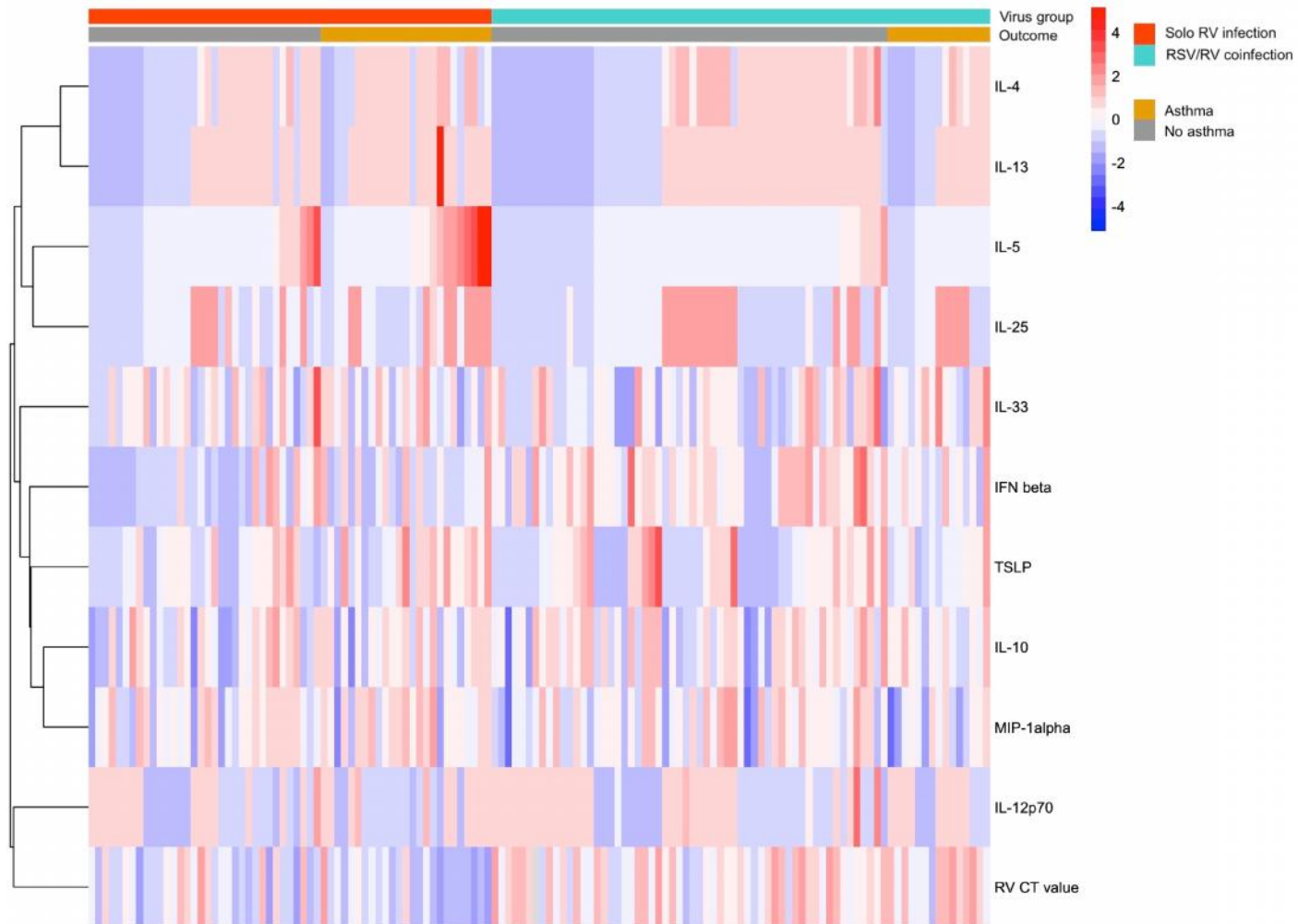


Figure E2. Association of nasopharyngeal cytokine levels in infants hospitalized for bronchiolitis with risk of asthma at age 4 years, according to virology

The fitted lines represent locally estimated scatterplot smoothed (loess) curve for infants with solo RV bronchiolitis and those with RV/RSV coinfection. **A)** IL-4, **B)** IL-5, **C)** IL-10, **D)** IL-12, **E)** IL-13, **F)** IL-25, **G)** IL-33, **H)** IFN- β , **I)** MIP-1 α , and **J)** TSLP levels. The grey bar represents the range in which 95% of data are present.

Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

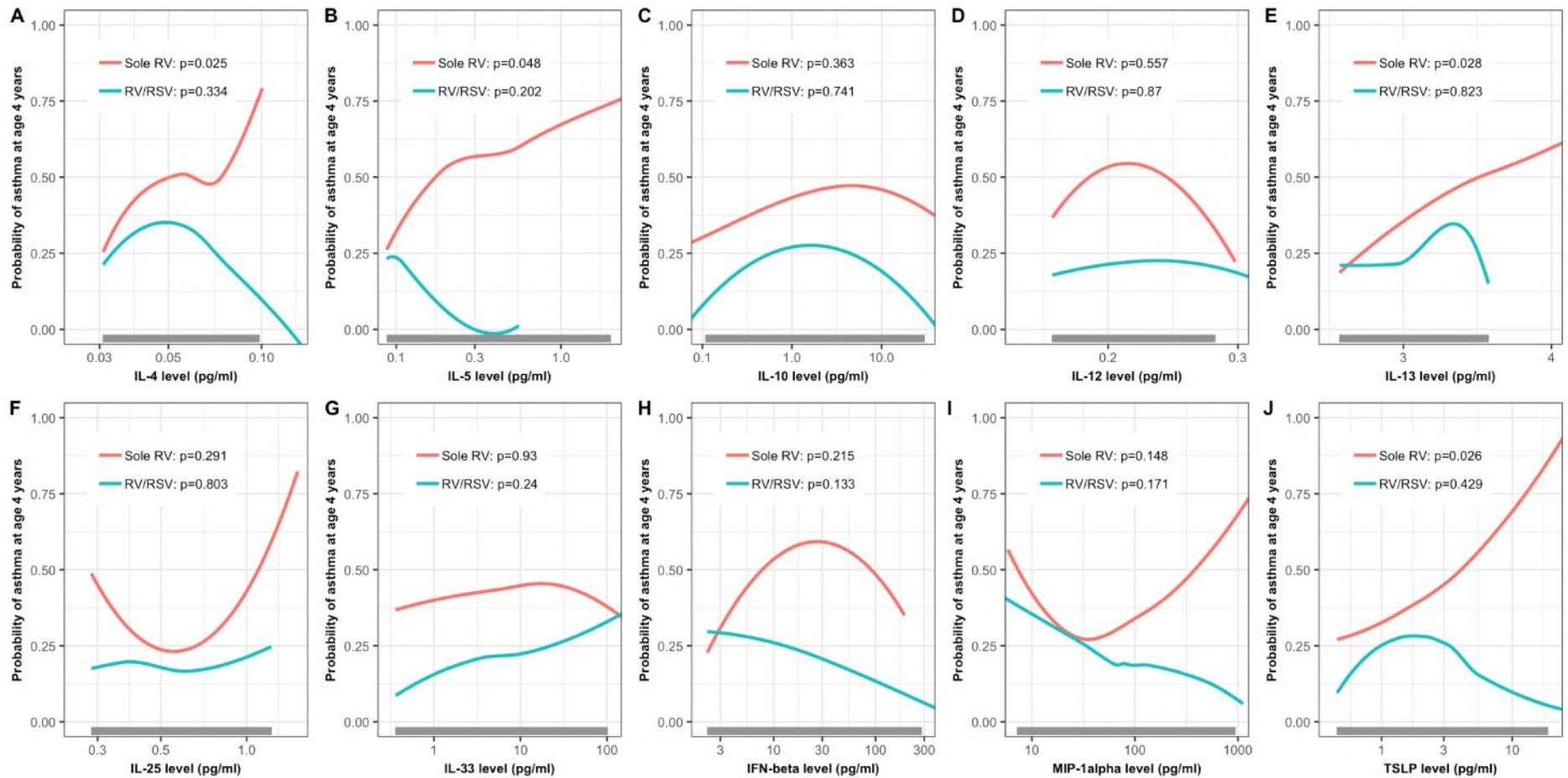


Figure E3. Association of normalized nasopharyngeal cytokine levels in infants hospitalized for bronchiolitis with risk of asthma at age 4 years, according to virology

The fitted lines represent locally estimated scatterplot smoothed (loess) curve for infants with solo RV bronchiolitis and those with RV/RSV coinfection. The levels of **A)** IL-4, **B)** IL-5, **C)** IL-10, **D)** IL-12, **E)** IL-13, **F)** IL-25, **G)** IL-33, **H)** IFN- β , **I)** MIP-1 α , and **J)** TSLP were normalized using RNase P copy number. The grey bar represents the range in which 95% of data are present. Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

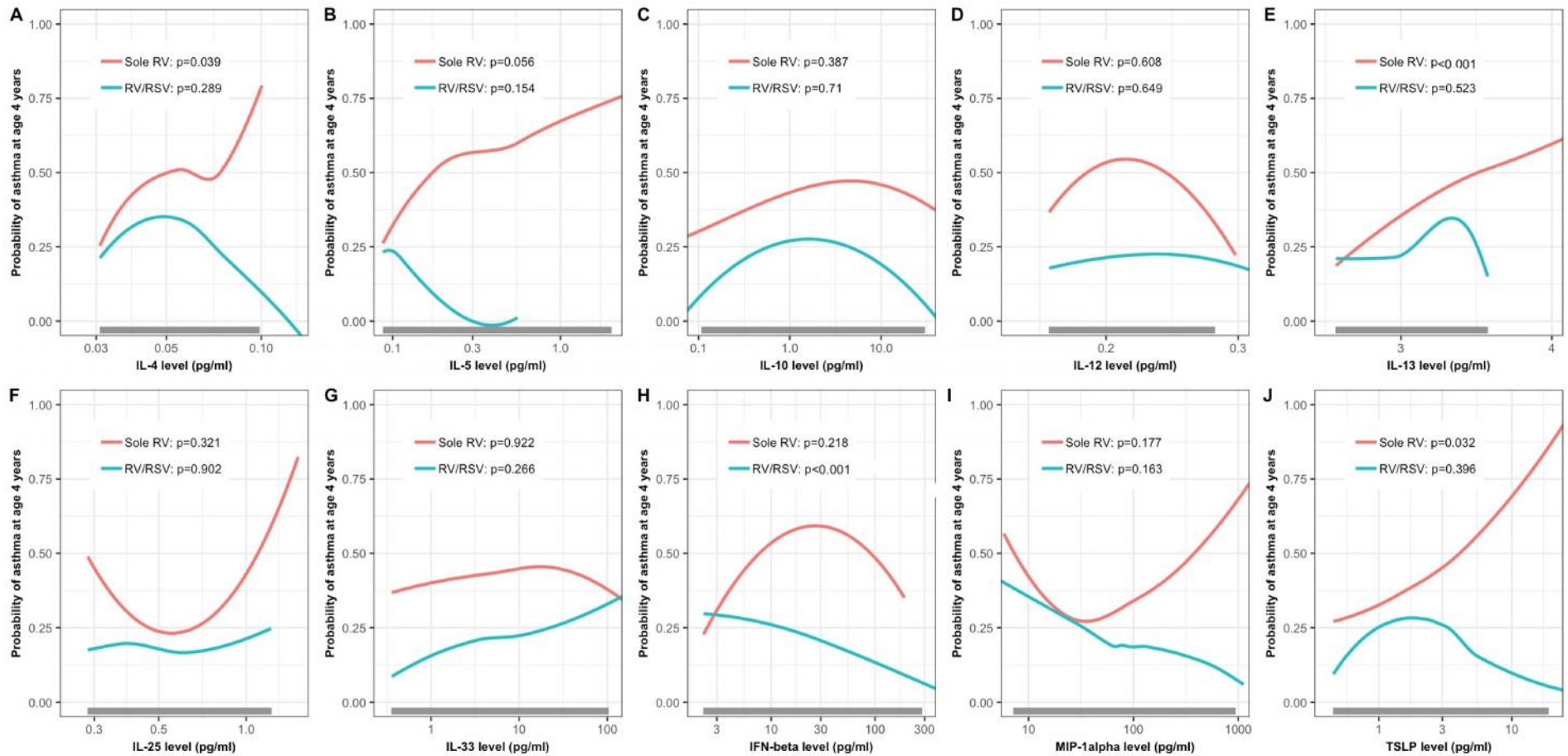


Figure E4. Association of nasopharyngeal cytokine levels in infants hospitalized for bronchiolitis with risk of asthma at age 4 years, excluding 37 infants who had a breathing problem prior to enrollment

The fitted lines represent locally estimated scatterplot smoothed (loess) curve for infants with solo RV bronchiolitis and those with RV/RSV coinfection. **A) IL-4, B) IL-5, C) IL-10, D) IL-12, E) IL-13, F) IL-25, G) IL-33, H) IFN- β , I) MIP-1 α , and J) TSLP levels.** The grey bar represents the range in which 95% of data are present. Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; MIP; macrophage inflammatory protein; RSV, respiratory syncytial virus; RV, rhinovirus; TSLP, thymic stromal lymphopoietin.

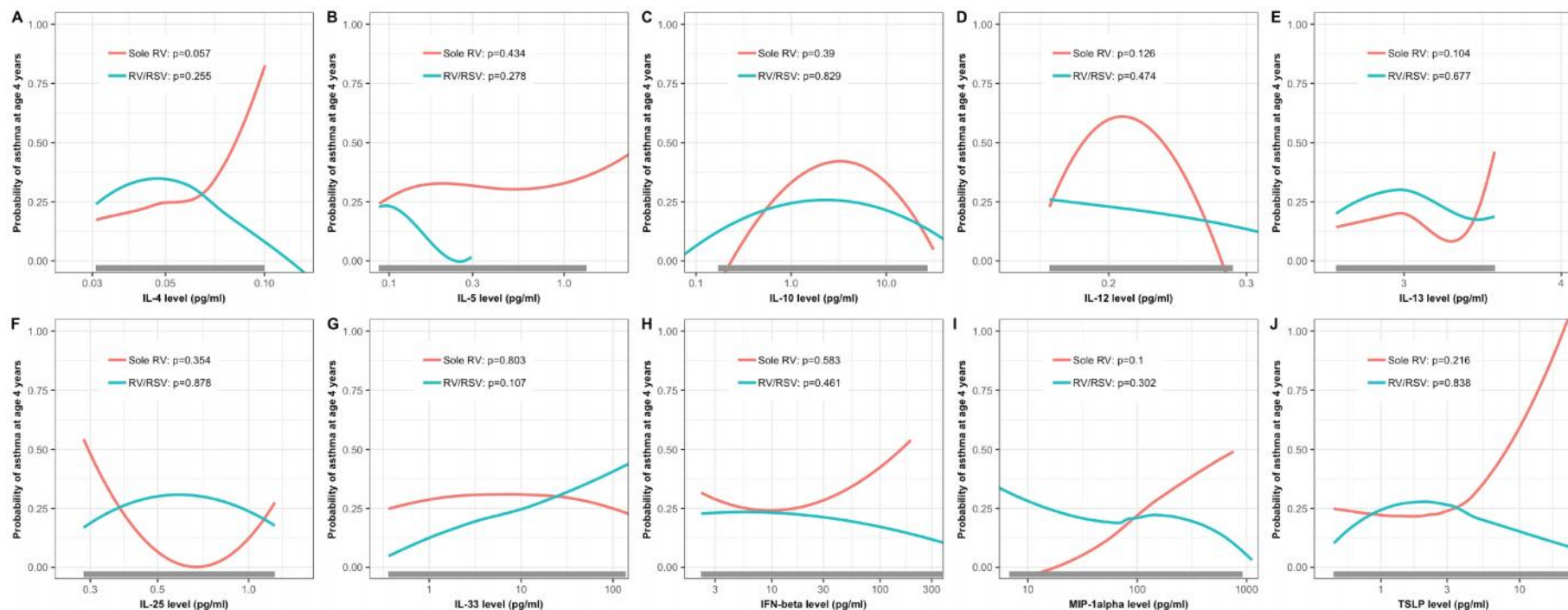


Figure E5. Correlations between nasopharyngeal airway interleukin-5 levels and blood eosinophil count in infants hospitalized with bronchiolitis

The fitted line represents locally estimated scatterplot smoothed (loess) curve with 95% CI. There was a significant correlation between IL-5 levels and blood eosinophil count ($\rho=0.27$, $P=0.01$).

