

MIP-1 α level in nasopharyngeal aspirates at the first wheezing episode predicts recurrent wheezing



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Background: Respiratory virus-induced wheezing, such as that induced by respiratory syncytial virus (RSV) and human rhinovirus, is an important risk factor for recurrent wheezing and childhood asthma. However, no biomarkers for predicting recurrent wheezing have been identified.

Objective: We searched for predictors of recurrent wheezing using nasopharyngeal aspirates obtained from patients during the first wheezing episode who were hospitalized with an acute lower respiratory tract illness.

Methods: We enrolled 82 infants during the first wheezing episode (median age, 5.0 months) who were hospitalized for acute lower respiratory tract illness between August 2009 and June 2012 and followed these patients for 2.5 years.

Nasopharyngeal aspirates and blood samples were obtained on the first day of hospitalization. Viral genomes were identified by using RT-PCR and sequencing. Levels of 33 cytokines, tryptase, IgE, anti-RSV IgE, and anti-RSV IgG were measured by using ELISAs or the Bio-Plex multiplex assay. Predictors of recurrent wheezing were examined by using a stepwise logistic regression model with backward elimination.

Results: Sixty percent of the patients experienced recurrent wheezing episodes. One or more viruses were detected in the nasopharynxes of 93% of the patients during the first wheezing episode. IFN- γ , IL-2, IL-9, MIP-1 α , and MIP-1 β levels were significantly higher among patients with recurrent wheezing than among those without recurrent wheezing ($P < .05$ or $.01$). The stepwise model demonstrated that the MIP-1 α level (odds ratio, 7.72; 95% CI, 1.50-39.77; $P = .015$) was the strongest independent predictor of the occurrence of recurrent wheezing. **Conclusion:** An increased MIP-1 α level in nasopharyngeal aspirates from patients with acute respiratory symptoms during the first wheezing episode caused by viral infections might predict recurrent wheezing. (J Allergy Clin Immunol 2016;137:774-81.)

Key words: Acute lower respiratory tract illness, biomarkers, human rhinovirus, recurrent wheezing, IFN- γ , MIP-1 α , MIP-1 β , nasopharyngeal aspirates, respiratory syncytial virus

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Viral infections of the lower respiratory tract are likely to result in wheezing if they induce inflammation and edema of the airway epithelium, decreasing airway diameter.¹ Lower respiratory tract infections, especially infection with respiratory syncytial virus (RSV), human rhinovirus (HRV), or both, induce early wheezing, which is an important risk factor for recurrent wheezing and childhood asthma.¹⁻⁸ Recurrent wheezing or childhood asthma has been reportedly linked to pronounced atopic characterization, low interferon responses, environmental factors (eg, maternal smoking and allergen exposure), and genetics (including a family history of asthma and allergic rhinitis).⁸⁻¹¹ However, no biomarkers for the prediction of recurrent wheezing are available for infants experiencing their first episode of wheezing.

Some evidence of viral specificity exists because allergic sensitization specifically increased the risk of wheezing in patients infected with HRV.^{9,10} Considering atopic characterization and genetics, we decided to measure total IgE, anti-RSV IgE, mast cell tryptase, and T_H2 cytokine levels in nasopharyngeal aspirates from infants during the first wheezing episode, and we collected information regarding complications related to food allergies, atopic dermatitis, and a family history of asthma.

Deficient interferon responses to HRV infection are reportedly present in asthmatic children and atopic patients.¹¹ Epithelial cell-derived cytokines, such as IL-33, IL-25, and thymic stromal lymphopoietin (TSLP), play important roles in the pathogenesis of bronchial asthma.¹² With regard to regulatory T cell-related cytokines, IL-10 is associated with post-RSV bronchiolitis-induced wheezing and asthma,^{13,14} and TGF- β enhances replication of RSV and HRV.^{15,16} Thus levels of T_H1 cytokines, including interferons, IL-33, IL-25, TSLP, TGF- β , and IL-10,

Abbreviations used

HBoV: Human bocavirus
HMPV: Human metapneumovirus
HRV: Human rhinovirus
RSV: Respiratory syncytial virus
TSLP: Thymic stromal lymphopoietin

in nasopharyngeal aspirates were measured in the present study. The possible presence of viral genomes, including RSV, HRV, human enterovirus, human metapneumovirus (HMPV), human parainfluenza virus, influenza virus, adenovirus, and human bocavirus (HBoV), in nasopharyngeal aspirates was identified by using RT-PCR. To identify biomarkers for prediction of recurrent wheezing, we measured a broad panel of inflammatory mediators in the nasopharyngeal aspirates of infants who were followed up for 2.5 years after their first wheezing episode.

METHODS

Ethical considerations

The study was approved by the ethics committees of all the affiliated institutions. All the subjects' guardians provided written informed consent in accordance with the Helsinki Declaration of the World Medical Association.

Patient enrollment

Patients were consecutively recruited between August 2009 and June 2012. The inclusion criteria included hospitalized infants with acute lower respiratory tract illness at the time of the first episode of wheezing and infants aged between 0.5 and 48 months and delivered at or after 36 weeks of gestation. These patients were treated with intravenous infusion of saline, supplemental oxygen, and β_2 -agonist or epinephrine nebulization. Exclusion criteria were the presence of an acute bacterial lower respiratory tract infection, a chronic nonatopic illness, and previous systemic or inhaled corticosteroid treatment. We enrolled 10 nonatopic outpatients who had the common cold without fever, lower respiratory tract symptoms, current wheezing, or a history of wheezing to measure basal levels of MIP-1 α in their nasopharyngeal aspirates.

Definitions and study protocol

Wheezing was defined as a high-pitched whistling sound during expiration with breathing difficulty.¹⁷ The patients were examined by pediatricians who clinically verified the wheezing and breathing difficulty. A medical and sociodemographic history was taken. On the first day of hospitalization for acute lower respiratory tract illness, nasopharyngeal aspirates/discharges were obtained for a viral diagnosis and measurement of mediators, and blood was drawn to measure blood cell counts and C-reactive protein, serum IgE, and house dust mite-specific IgE levels. In some patients an RSV antigen rapid-diagnosis kit (ImmunoCard STAT! RSV PLUS Test kit; Cardinal Health, Dublin, Ohio) was used to check for RSV infection. All samples, including nasopharyngeal aspirates and blood, were obtained before treatment. Our definition of laboratory findings indicative of bacterial lower respiratory tract infection is a peripheral blood white blood cell count of more than 15,000/ μ L, a serum C-reactive protein level of more than 10 mg/dL, and the presence of infiltrative opacities on the chest radiograph.

Patient follow-up and end point

For infants with respiratory symptoms, medical examinations by interviews with the parents were scheduled for 1 month, 3 months, 6 months, 1 year, and 2.5 years after hospitalization and conducted by means of examination in the outpatient clinics or by means of telephone. The patients were followed up for 2.5 years after the first wheezing episode. Recurrent wheezing was defined as

the occurrence of 2 or more episodes of expiratory wheezing diagnosed by a pediatrician in the outpatient clinic. When patients visited doctors or emergency departments for intercurrent illnesses, the patients' parents were asked by telephone whether wheezing had been diagnosed by the treating pediatrician. Pediatricians' records were reviewed for all intercurrent doctor's office visits, emergency department visits, and hospitalizations for respiratory symptoms.

DNA/RNA extraction, PCR, and sequencing

An advanced flocked swab (Copan Diagnostics, Murrieta, Calif) was used to collect nasopharyngeal discharges, and the samples were stored in Universal Transport Medium (Copan Innovation, Brescia, Italy) at -80°C . For viral DNA/RNA extraction, RT-PCR, and sequence analysis, the nasopharyngeal swab samples were centrifuged at 3000g and 4°C for 15 minutes, and the resulting supernatants were used for RT-PCR and sequence analysis, as described previously (see the [Methods](#) section in this article's Online Repository at www.jacionline.org for additional information regarding the detailed experimental protocols).^{18,19}

Processing of nasopharyngeal aspirates and measurement of cytokine and tryptase concentrations

Nasopharyngeal aspirates were processed with 1 volume of 0.1% dithiothreitol (Sputolysin; Calbiochem, Bad Soden, Germany) for 15 minutes at room temperature. The dithiothreitol-treated nasopharyngeal aspirates were filtered through a 53- μ m nylon mesh and centrifuged (700g, 10 minutes, 4°C). Supernatants were frozen at -80°C . The remaining cell pellets were resuspended in PBS. Cell counts were performed with a hemocytometer, and viability was determined by using trypan blue solution (Sigma, Deisenhofen, Germany). Cytospins were prepared, and differential cell counts were performed with hematoxylin and eosin staining. We measured the following 29 cytokines with an available kit (Cytokine assay Bio-Plex Pro 27-Plex Panel kit and IL-25 and IL-33 sets; Bio-Rad Laboratories, Hercules, Calif): IL-1 β , IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IL-25, IL-33, eotaxin, fibroblast growth factor basic, granulocyte colony-stimulating factor, GM-CSF, IFN- γ , interferon-inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein (MIP) 1 α , MIP-1 β , platelet-derived growth factor-BB, RANTES, TNF- α , and vascular endothelial growth factor. Assays were performed with the Bio-Plex suspension array system, according to the manufacturer's instructions (Bio-Rad). Data were automatically processed and analyzed by using Bio-Plex Manager Software 5.0 with the standard curve produced from the recombinant cytokine standard. The sensitivity limits for each cytokine were set at 0.1 to 3 pg/mL.^{20,21} TGF- β levels were measured with ELISA kits purchased from R&D Systems (Minneapolis, Minn). Sensitivity limits for TGF- β were set at 1.7 to 15.4 pg/mL. IFN- α and IFN- λ ELISA kits were purchased from PBL Assay Science (Piscataway, NJ), and the sensitivity limits for IFN- α and IFN- λ were set at 12.5 and 62.5 pg/mL, respectively. The TSLP ELISA kit was purchased from MyBioSource (San Jose, Calif; sensitivity, 5.1 pg/mL). Tryptase levels were determined by using ImmunoCAP tryptase (Thermo Fisher Scientific, Uppsala, Sweden; sensitivity, 1.0 ng/mL).

Measurement of concentrations of total IgE, anti-RSV IgE, and anti-RSV IgG in nasopharyngeal aspirates

The human IgE ELISA quantitation kit (CosmoBio, Tokyo, Japan) was used to measure total IgE levels. The sensitivity limit for IgE was set at 15.6 ng/mL. Microtiter plates were coated with synthesized RSV G protein (1 μ g/mL) to measure anti-RSV IgE and anti-RSV IgG levels (see the [Methods](#) section in this article's Online Repository for additional information regarding experimental protocols). The plates were then incubated with 1% BSA-PBS at room temperature for 1 hour, washed, and then incubated with

TABLE I. Comparison of characteristics between patients with and without recurrent wheezing episodes

Characteristic	Total, n = 82	RW (–), n = 33	RW (+), n = 49	RW (–) vs RW (+), <i>P</i> value*
Age at first episode of wheeze (mo), median (minimum-maximum)	5.0 (0.5-40.0)	4.5 (1.0-30.0)	6.0 (0.5-40.0)	.296†
Male sex (%)	59	58	59	1.000*
Family history of asthma (%)	45	49	41	.656*
Siblings or day care (%)	79	70	85	.229*
Doctor-diagnosed atopic dermatitis (%)	8	3	11	.392*
Doctor-diagnosed food allergy (%)	14	6	19	.063*
Pets at home (%)	14	10	16	.402*
Parental smoking (%)	28	35	24	.538*
Clinical characteristics on admission				
Tachypnea (%)	37	36	38	1.000*
Retracted breathing (%)	19	22	23	.259*
Oxygen saturation (%), median (minimum-maximum)	97 (90-100)	97 (90-100)	98 (90-100)	.139†
Pco ₂ (mm Hg), median (minimum-maximum)	38 (24-59)	38 (27-58)	38 (24-59)	.927†
Serum CRP level (mg/dL), median (minimum-maximum)	0.6 (0.0-8.9)	0.7 (0.0-8.9)	0.5 (0.0-7.7)	.673†
Peripheral leukocyte counts (/μL), median (minimum-maximum)	9,850 (5,200-15,460)	9,900 (5,200-15,000)	9,800 (5,200-15,460)	.669†
Blood eosinophils (/μL), median (minimum-maximum)	116 (0-1,422)	92 (0-1,422)	137 (0-570)	.772†
Serum IgE level (IU/L), median (minimum-maximum)	38.5 (2.6-531.0), n = 32	33.9 (4.7-531.0), n = 11	46.2 (2.6-232.6), n = 21	.843†
House dust mite-specific IgE level (UA/mL), median (minimum-maximum)	0.17 (0.17-86.50), n = 16	12.94 (0.17-86.50), n = 4	0.17 (0.17-26.80), n = 12	.379†

CRP, C-reactive protein; RW (+), patients with recurrent wheezing episodes; RW (–), patients without recurrent wheezing episodes.

*Fisher exact test.

†Mann-Whitney *U* test.

nasopharyngeal aspirates (diluted 1:2) at room temperature for 2 hours. After incubation with biotin-conjugated anti-human IgE or anti-human IgG (BD Biosciences, Tokyo, Japan) and washing, plates were incubated with streptavidin-peroxidase (R&D Systems) at room temperature for 30 minutes. The colorimetric signal of tetramethylbenzidine was detected by measuring absorbance at 450/570 nm with a plate reader.

Statistical analysis

To identify the variables associated with recurrent wheezing, we compared continuous variables between patients with and without recurrent wheezing using the Mann-Whitney *U* test. For comparison of categorical variables, a 2-sided Fisher exact test was used with a prespecified significance level of .5. Predictors of recurrent wheezing were examined by using a stepwise logistic regression model with backward elimination. First, variables with a significance level of less than .20 in univariate analysis were included in a multiple logistic regression. If the same variable was selected when both the original raw value and the log-transformed value were used, we selected the normally distributed variable. Finally, we performed a logistic regression with backward elimination and estimated effects on the odds ratio. Spearman rank correlation coefficients were calculated to determine the strength of the correlations between continuous variables. Kruskal-Wallis tests were used for comparing differences between 3 patient groups.

See the [Methods](#) section in this article's Online Repository for additional information regarding the experimental protocols.

RESULTS

Recruitment and baseline characteristics

We enrolled 82 infants according to the inclusion and exclusion criteria described in the [Methods](#) section. The baseline characteristics are shown in [Table I](#). Forty-five percent of the patients had a family history of asthma. Atopic dermatitis and

food allergy complications were observed in 8% and 14% of patients, respectively. The median C-reactive protein level was 0.6 mg/dL (minimum-maximum, 0.0-8.9 mg/dL).

Comparison of characteristics between patients with and without recurrent wheezing episodes

Sixty percent of patients experienced recurrent wheezing episodes. The frequency of food allergy complications tended to be higher in patients with recurrent wheezing episodes than in those without wheezing episodes (*P* = .063, Fisher exact test), although the difference was not statistically significant in the frequencies of atopic dermatitis complications, the frequencies of a family history of bronchial asthma, and clinical data, including oxygen saturation, leukocyte counts, eosinophil counts, and serum IgE levels, observed between these patients ([Table I](#)). The differential cell counts in nasopharyngeal aspirates from patients with and those without recurrent wheezing episodes are shown in [Table E1](#) in this article's Online Repository at www.jacionline.org. No significant differences in differential cell counts were observed between the 2 patient groups.

Viral cause in patients with their first wheezing episode who were hospitalized for acute lower respiratory tract illness

Of the 82 enrolled patients, RT-PCR and a viral nucleotide sequence analysis were performed in 60, whereas an RSV antigen rapid-diagnosis kit was used to test for RSV infection in 18 ([Table II](#)). The final 4 patients did not undergo either an RT-PCR analysis or RSV antigen rapid diagnosis. One or more viruses

TABLE II. Viral cause in patients hospitalized for acute respiratory symptoms with their first episode of wheezes

Virus detected	Total, n = 60, no. (%)	RT-PCR		RSV antigen Rapid-diagnosis kit, no.
		RW (–), n = 26, no. (%)	RW (+), n = 34, no. (%)	
RSV	40 (66.7)	22 (84.6)	19 (55.9)	18
HRV	20 (33.3)	7 (26.9)	13 (38.2)	
Metapneumovirus	4 (6.7)	0 (0.0)	4 (11.8)	
HBoV	2 (3.3)	0 (0.0)	2 (58.8)	
Human parainfluenza virus	0 (0.0)	0 (0.0)	0 (0.0)	
Adenovirus	0 (0.0)	0 (0.0)	0 (0.0)	
Enterovirus	0 (0.0)	0 (0.0)	0 (0.0)	
Influenza virus	0 (0.0)	0 (0.0)	0 (0.0)	
One virus	45 (75.0)	21 (80.8)	24 (70.6)	
Two viruses	11 (18.3)	4 (15.4)	7 (20.6)	
Not detected	4 (6.7)	1 (3.8)	3 (8.8)	

RW (+), Patients with recurrent wheezing episodes; RW (–), patients without recurrent wheezing episodes.

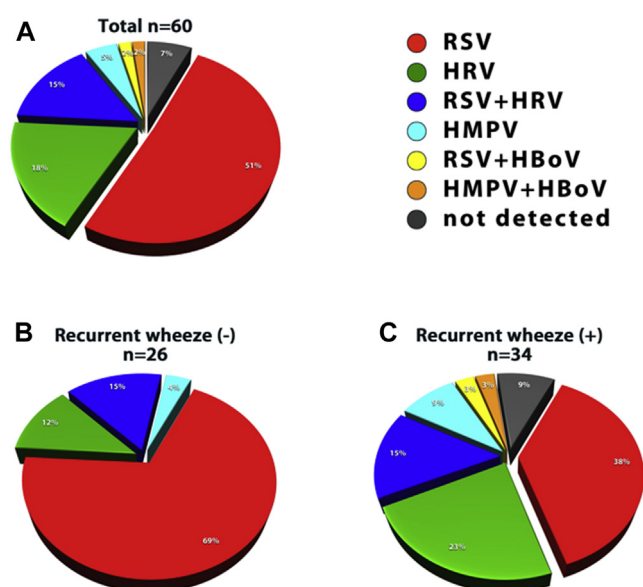


FIG 1. Comparison of viral cause between patients with and without recurrent wheezing episodes. Viral cause is shown for all patients with their first wheezing episode who were hospitalized for acute lower respiratory tract illness (n = 60, **A**) and separately for patients without (**B**) and with (**C**) recurrent wheezing.

were detected in the nasopharynxes of 93% of patients with their first wheezing episode (56/60 [93%], **Fig 1**). Four types of PCR-positive viruses were detected in the 56 (93%) patients with positive results (**Fig 1** and **Table II**). Among them, the most common was RSV (51%), followed by HRV (18%), codetection of RSV and HRV (15%), HMPV (5%), codetection of RSV and HBoV (2%), and codetection of HMPV and HBoV (2%; **Fig 1, A**). Overall, RSV or HRV was detected in the nasopharynxes of 86% of the patients with their first wheezing episode.

Comparison of viral cause in patients with and without recurrent wheezing episodes

As shown in **Fig 1, B**, RSV alone, HRV alone, and codetection of both RSV and HRV were detected in the nasopharynxes of 69%, 12%, and 15% of patients with their first wheezing episode who did not experience recurrent wheezing over a period of 2.5

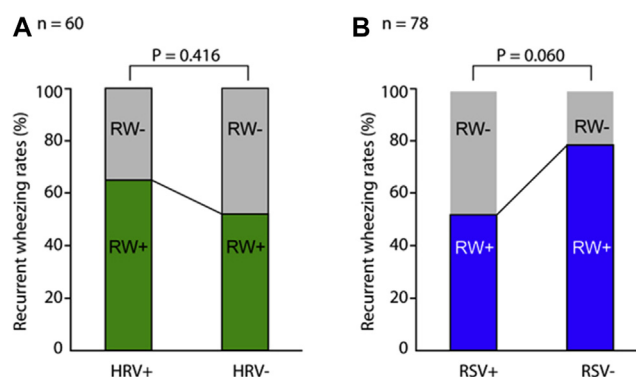


FIG 2. Comparison of recurrent wheezing rates between patients who had HRV (HRV^+) and those who did not (HRV^- , **A**) and between patients who had RSV (RSV^+) and those who did not (RSV^- , **B**).

years, respectively. RSV alone, HRV alone, and codetection of both were detected in the nasopharynxes of 38%, 23%, and 15% of patients with their first wheezing episode who experienced recurrent wheezing, respectively (**Fig 1, C**). We compared recurrent wheezing rates between patients with positive and negative test results for HRV in the nasopharynx (HRV^+ and HRV^- , respectively) and those with positive and negative test results for RSV (RSV^+ and RSV^- , respectively). No significant differences in recurrent wheezing rates were observed between the HRV^+ and HRV^- groups (**Fig 2, A**) or between the RSV^+ and RSV^- groups (**Fig 2, B**).

Prediction of recurrent wheezing episodes

Cytokine, chemokine, tryptase, anti-RSV G protein IgE, anti-RSV G protein IgG, and total IgE levels in nasopharyngeal aspirates were compared between patients with and those without recurrent wheezing. IFN- γ , IL-2, IL-9, MIP-1 α , and MIP-1 β levels were significantly higher in nasopharyngeal aspirates from patients who had experienced recurrent wheezing than in nasopharyngeal aspirates from patients who had not experienced recurrent wheezing ($P < .05$ or $.01$, Mann-Whitney U test; **Figs 3** and **4**). We used logistic regression models to predict the occurrence of recurrent wheezing using associated variables with backward elimination. A multiple stepwise regression analysis with all the predictors identified 14 of them (see **Table E2** in this article's Online Repository at www.jacionline.org).

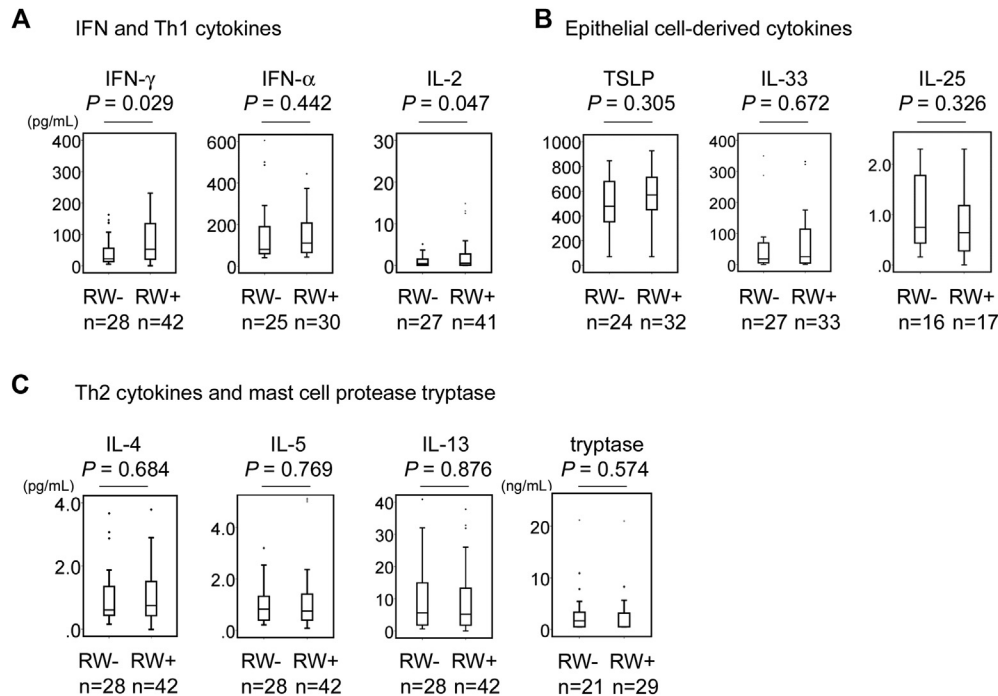


FIG 3. Comparison of interferon and T_H1 cytokine (A), epithelial cell-derived cytokine (B), and T_H2 cytokine and mast cell protease tryptase (C) levels in nasopharyngeal aspirates from patients with and those without recurrent wheezing (RW) episodes. Median values and interquartile ranges are shown. Values for cytokine and tryptase levels are shown in picograms per milliliter and nanograms per milliliter, respectively.

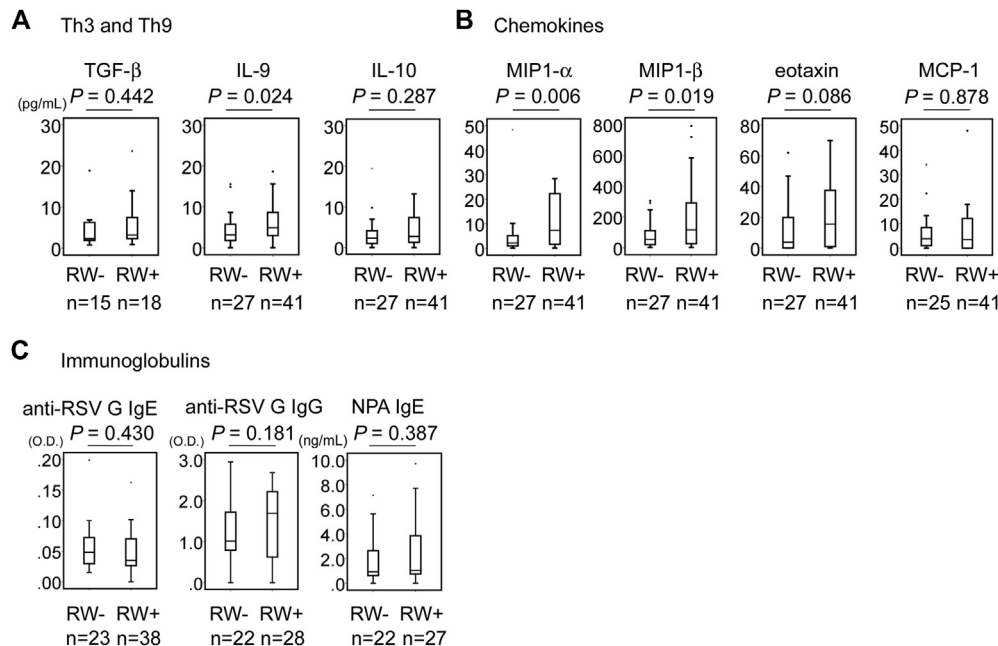


FIG 4. Comparison of T_H3 and T_H9 cytokine (A), chemokine (B), and immunoglobulin (C) levels in nasopharyngeal aspirates from patients with and those without recurrent wheezing (RW) episodes. Medians and interquartile ranges are shown. Units for plasma cytokine levels and nasopharyngeal aspirate (NPA) IgE levels are shown in picograms per milliliter and nanograms per milliliter, respectively. Anti-RSV G IgE and anti-RSV G IgG levels are shown as optical density (O.D.) values.

Logistic regression with backward elimination using these 14 identified predictors included MIP-1 α (odds ratio, 7.72; 95% CI, 1.50-39.77; $P = .015$), IL-9, IL-15, and nasopharyngeal

aspirate total IgE (Table III). The MIP-1 α level was the strongest independent predictor of recurrent wheezing episodes. As expected, the MIP-1 α level was significantly correlated with

TABLE III. Logistic regression with backward elimination

Predictive variable	P value	OR	95% CI	
			Lower	Upper
Log ₁₀ MIP-1 α	.015	7.72	1.50	39.77
Log ₁₀ IL-9	.099	0.13	0.01	1.46
IL-15	.122	1.06	0.99	1.13
NPA total IgE	.205	1.43	0.82	2.47

NPA, Nasopharyngeal aspirate; OR, odds ratio.

IFN- γ ($r = 0.508$), MIP-1 β ($r = 0.694$), IL-2 ($r = 0.377$), and IL-9 ($r = 0.512$) levels ($P < .05$ for all, Spearman rank correlation coefficient; see [Table E3](#) in this article's Online Repository at www.jacionline.org).

DISCUSSION

In this study we demonstrated that increased MIP-1 α levels in the nasopharyngeal aspirates of infants with their first episode of wheezing associated with an acute lower respiratory viral infection might be predictive of the occurrence of recurrent wheezing. MIP-1 α , also called chemokine (C-C motif) ligand 3, is produced by macrophages, dendritic cells, and lymphocytes and exhibits chemotactic properties for eosinophils, monocytes, and lymphocytes.²² MIP-1 α has been implicated in airway inflammation related to asthma, and it plays a key role in driving the late-phase allergic reaction²³⁻²⁵ through eosinophil infiltration.²⁶⁻²⁸ Eosinophils play a key role in tissue remodeling in murine asthma models.²⁹ MIP-1 α itself also induces airway tissue remodeling through airway smooth muscle proliferation and survival.³⁰ Infection with RSV³¹ and HRV³² increases MIP-1 α levels in the airways. In MIP-1 α knockout or anti-MIP-1 α -treated RSV-infected mice, more RSV-specific proinflammatory T cells were recruited to the lungs of both types of mice when MIP-1 α responses were impaired, whereas fewer total T cells (CD4⁺ T cells plus CD8⁺ T cells) were recruited to the lungs of both mice. This increase in RSV-specific proinflammatory T-cell counts was accompanied by increased weight loss and illness after RSV infection.³³ Furthermore, primary RSV infection in allergen-sensitized mice transiently increases airway responsiveness, which is accompanied by increases in eosinophilic infiltration and MIP-1 α levels in lung tissue³⁴ and bronchoalveolar fluid.³⁵ A secondary RSV infection persistently enhanced airway responsiveness in allergen-sensitized mice, with a concomitant increase in MIP-1 α levels in lung tissues.³⁴

These data indicate that MIP-1 α induced by both viral respiratory tract infection and bronchial asthma can be implicated in airway inflammation, hyperresponsiveness, and remodeling. We measured MIP-1 α levels in the nasopharyngeal aspirates of 10 nonatopic outpatients aged a median of 12 months (minimum–maximum, 3–19 months) who had the common cold without fever, lower respiratory tract symptoms, current wheezing, or a history of wheezing as reference. A statistically significant difference among 3 patient groups was found regarding MIP-1 α levels in nasopharyngeal aspirates of outpatients with the common cold (median, 0.05 pg/mL; interquartile range, 0.05–17.36 pg/mL) and hospitalized patients with (median, 7.30 pg/mL; interquartile range, 1.56–22.60 pg/mL) and without (median, 2.15 pg/mL; interquartile range, 0.93–5.56 pg/mL) recurrent wheezing ($P = .001$, Kruskal-Wallis test).

IFN- γ concentrations in samples of blood mononuclear cells or nasopharyngeal aspirates from RSV-infected infants, HRV-infected infants, or both were inversely correlated with disease severity,³⁶⁻⁴² suggesting that IFN- γ plays an important role in determining the severity of RSV-induced bronchiolitis, HRV-induced bronchiolitis, or both. In mice IFN- γ production during primary RSV infection has been reported to be critical to the development of protection against airway hyperresponsiveness and airway eosinophilia, as well as mucus hyperproduction, during subsequent reinfection.⁴³ However, our data showed that IFN- γ levels were significantly higher in nasopharyngeal aspirates from patients who had experienced recurrent wheezing than in nasopharyngeal aspirates from patients who had not experienced recurrent wheezing. Thus the severity of RSV-induced bronchiolitis, HRV-induced bronchiolitis, or both at the time of the first wheezing episode does not determine the recurrent wheezing outcome. Data from adults and children with asthma indicate that innate antiviral responses (eg, production of IFN- α , IFN- β , and IFN- λ) might be deficient in the airways of asthmatic patients and might be instrumental in explaining the susceptibility of the asthmatic population to wheezing attacks caused by HRV and possibly other viral pathogens.^{11,14,44-46} In contrast, IFN- λ levels are reportedly higher in wheezing children infected with HRV compared with those in nonwheezing children and increase as symptoms worsen.⁴⁷ Our results show that there was no significant difference in IFN- α levels between patients with and without recurrent wheezing. IFN- λ levels in nasopharyngeal aspirates from patients were less than the ELISA detection limits in most cases. Thus the low responses to respiratory tract viruses in asthmatic patients were not seen in patients with wheezing illness at the time of the first wheezing episode.

Total and specific serum IgE levels, eczema in the patient, and a parental history of atopy were reportedly not associated with wheezing.⁴⁸ These findings agree with our present results. Sensitization to aeroallergens beginning during the first year of life consistently predisposes children to HRV-induced wheezing illnesses, but the converse is not true.¹⁰ Although we were unable to examine sensitization to aeroallergens in all patients, we found that the frequency of food allergy complications tended to be higher in patients with recurrent wheezing episodes compared with values in those without wheezing episodes ($P = .063$, [Table I](#)), suggesting that sensitization to food allergens before respiratory virus-induced wheezing illness might be a risk factor for recurrent wheezing. In animal models RSV-specific IgE enhanced airway responsiveness on reinfection with RSV in newborns.⁴⁹ In our data no significant differences in RSV-specific IgE levels in nasopharyngeal aspirates were observed between patients with and without recurrent wheezing.

Many previous reports have highlighted wheezing induced by HRV in young children as an important predictor of recurrent wheezing.^{7,19,50} For example, Fujitsuka et al¹⁹ showed that recurrent wheezing in children with acute respiratory tract infection tends to be induced by HRV, whereas the first episode of wheezing was induced by RSV. In the present study no significant difference in the percentage of patients with recurrent wheezing was observed between the HRV⁺ and HRV[−] groups ([Fig 2, A](#)) or between the RSV⁺ and RSV[−] groups ([Fig 2, B](#)). In line with our findings, 2 different birth cohort studies have also reported that the association between respiratory tract infections in early life and asthma in later life was independent

of the type of virus causing the respiratory tract infection; that is, there was no statistically significant difference in the risk of recurrent wheezing between infants with respiratory tract infections caused by the 2 types of viruses.^{5,51} This discrepancy might be due to the differences in the prevalence of each virus in each season.^{19,52}

The study has some limitations. First, the study sample was restricted to hospitalized infants. Therefore the sample is limited to enrollees with relatively severe clinical illness, limiting the external validity of the results to a broader sample of infants, such as those with milder clinical illnesses.

Second, this is a hypothesis-generating study, and the findings remain to be confirmed in a different population. To identify the cutoff value of MIP-1 α , we examined the dose-response relationship between the “linear values of MIP-1 α ” and the “frequency of occurrence of recurrent wheezing” by using a receiver operating characteristic curve. However, we could still not estimate a definite cutoff value of MIP-1 α because of the small number of patients and paucity of previous reports to verify the validity of the cutoff value. The tentative cutoff value of LogMIP-1 α was 0.396, which yielded a sensitivity of 68.3% and specificity of 59.3%. We propose to carry out a cohort study in the future with enrollment of more than 200 other patients to identify a definite cutoff value.

Administration of palivizumab, a humanized mAb that targets the A antigenic site of the fusion protein of RSV, decreases the risk of hospitalization in high-risk infants, as well as the risk of recurrent wheezing.^{53,54} Although we did not measure viral load in the present study, prednisolone might be beneficial in the subgroup of children with high viral loads.¹⁷ Treatment with the leukotriene receptor antagonist montelukast reduces eosinophil degranulation and eosinophil-derived neurotoxin levels and is associated with a decrease in recurrent wheezing episodes in patients with post-RSV bronchiolitis.⁵⁵ Furthermore, collection of samples of nasopharyngeal aspirates from infants is easy, even in the outpatient clinic setting, and an assay kit for MIP-1 α is commercially available. Thus measurement of MIP-1 α levels in nasopharyngeal aspirates from patients with their first wheezing episode with acute respiratory symptoms might be useful for predicting recurrent wheezing and initiating early intervention, such as treatment with steroids and leukotriene receptor antagonists or administration of palivizumab.

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Clinical implications: Measurement of MIP-1 α levels in nasopharyngeal aspirates from patients with their first wheezing episodes with acute lower respiratory tract illness might be useful for predicting recurrent wheezing and initiating early intervention.

REFERENCES

1. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. *Lancet* 2008;372:1100-6.
2. Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501-7.
3. Schauer U, Hoffjan S, Bittscheidt J, Kochling A, Hemmis S, Bongartz S, et al. RSV bronchiolitis and risk of wheeze and allergic sensitisation in the first year of life. *Eur Respir J* 2002;20:1277-83.
4. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;178:667-72.
5. Kusel MM, de Klerk NH, Keadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;119:1105-10.
6. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. *Lancet* 2008;372:1058-64.
7. Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;116:571-7.
8. Caliskan M, Bochkov YA, Kreiner-Moller E, Bonnelykke K, Stein MM, Du G, et al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013;368:1398-407.
9. Jartti T, Kausipalo H, Vuorinen T, Soderlund-Venermo M, Allander T, Waris M, et al. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatr Allergy Immunol* 2010;21:1008-14.
10. Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Pappas TE, Lee WM, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med* 2012;185:281-5.
11. Baraldo S, Contoli M, Bazzan E, Turato G, Padovani A, Marku B, et al. Deficient antiviral immune responses in childhood: distinct roles of atopy and asthma. *J Allergy Clin Immunol* 2012;130:1307-14.
12. Lloyd CM, Saglani S. Epithelial cytokines and pulmonary allergic inflammation. *Curr Opin Immunol* 2015;34C:52-8.
13. Schuurhof A, Janssen R, de Groot H, Hodemaekers HM, de Klerk A, Kimpen JL, et al. Local interleukin-10 production during respiratory syncytial virus bronchiolitis is associated with post-bronchiolitis wheeze. *Respir Res* 2011;12:121.
14. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Keadze T, et al. Rhinovirus-induced lower respiratory tract illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A* 2008;105:13562-7.
15. McCann KL, Imani F. Transforming growth factor beta enhances respiratory syncytial virus replication and tumor necrosis factor alpha induction in human epithelial cells. *J Virol* 2007;81:2880-6.
16. Bedke N, Sammut D, Green B, Kehagia V, Dennison P, Jenkins G, et al. Transforming growth factor-beta promotes rhinovirus replication in bronchial epithelial cells by suppressing the innate immune response. *PLoS One* 2012;7:e44580.
17. Jartti T, Nieminen R, Vuorinen T, Lehtinen P, Vahlberg T, Gern J, et al. Short- and long-term efficacy of prednisolone for first acute rhinovirus-induced wheezing episode. *J Allergy Clin Immunol* 2015;135:691-8.e9.
18. Mizuta K, Hirata A, Suto A, Aoki Y, Ahiko T, Itagaki T, et al. Phylogenetic and cluster analysis of human rhinovirus species A (HRV-A) isolated from children with acute respiratory infections in Yamagata, Japan. *Virus Res* 2010;147:265-74.
19. Fujitsuka A, Tsukagoshi H, Arakawa M, Goto-Sugai K, Ryo A, Okayama Y, et al. A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness. *BMC Infect Dis* 2011;11:168.
20. Okazaki K, Kondo M, Kato M, Kakinuma R, Nishida A, Noda M, et al. Serum cytokine and chemokine profiles in neonates with meconium aspiration syndrome. *Pediatrics* 2008;121:e748-53.
21. Yoshizumi M, Nakamura T, Kato M, Ishioka T, Kozawa K, Wakamatsu K, et al. Release of cytokines/chemokines and cell death in UVB-irradiated human keratinocytes, HaCaT. *Cell Biol Int* 2008;32:1405-11.
22. Maurer M, von Stebut E. Macrophage inflammatory protein-1. *Int J Biochem Cell Biol* 2004;36:1882-6.
23. Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. *Am J Respir Crit Care Med* 1997;156:1377-83.
24. Bisset LR, Schmid-Grendelmeier P. Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. *Curr Opin Pulm Med* 2005;11:35-42.
25. Lim S, John M, Seybold J, Taylor D, Witt C, Barnes PJ, et al. Increased interleukin-10 and macrophage inflammatory protein-1 alpha release from blood monocytes ex vivo during late-phase response to allergen in asthma. *Allergy* 2000;55:489-95.

26. Lukacs NW, Standiford TJ, Chensue SW, Kunkel RG, Strieter RM, Kunkel SL. C-C chemokine-induced eosinophil chemotaxis during allergic airway inflammation. *J Leukoc Biol* 1996;60:573-8.
27. Lukacs NW, Strieter RM, Warmington K, Lincoln P, Chensue SW, Kunkel SL. Differential recruitment of leukocyte populations and alteration of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. *J Immunol* 1997;158:4398-404.
28. Campbell EM, Kunkel SL, Strieter RM, Lukacs NW. Temporal role of chemokines in a murine model of cockroach allergen-induced airway hyperreactivity and eosinophilia. *J Immunol* 1998;161:7047-53.
29. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305:1776-9.
30. Halwani R, Al-Abri J, Beland M, Al-Jahdali H, Halayko AJ, Lee TH, et al. CC and CXC chemokines induce airway smooth muscle proliferation and survival. *J Immunol* 2011;186:4156-63.
31. Foronjy RF, Dabo AJ, Cummins N, Geraghty P. Leukemia inhibitory factor protects the lung during respiratory syncytial viral infection. *BMC Immunol* 2014;15:41.
32. Rajan D, McCracken CE, Kopleman HB, Kyu SY, Lee FE, Lu X, et al. Human rhinovirus induced cytokine/chemokine responses in human airway epithelial and immune cells. *PLoS One* 2014;9:e114322.
33. Tregoning JS, Pribul PK, Pennycook AM, Hussell T, Wang B, Lukacs N, et al. The chemokine MIP1alpha/CCL3 determines pathology in primary RSV infection by regulating the balance of T cell populations in the murine lung. *PLoS One* 2010;5:e9381.
34. Matsuse H, Behera AK, Kumar M, Rabb H, Lockey RF, Mohapatra SS. Recurrent respiratory syncytial virus infections in allergen-sensitized mice lead to persistent airway inflammation and hyperresponsiveness. *J Immunol* 2000;164:6583-92.
35. Ishioka T, Yamada Y, Kimura H, Yoshizumi M, Tsukagoshi H, Kozawa K, et al. Elevated macrophage inflammatory protein 1alpha and interleukin-17 production in an experimental asthma model infected with respiratory syncytial virus. *Int Arch Allergy Immunol* 2013;161(suppl 2):129-37.
36. Aberle JH, Aberle SW, Dworzak MN, Mandl CW, Rebhandl W, Vollnhöfer G, et al. Reduced interferon-gamma expression in peripheral blood mononuclear cells of infants with severe respiratory syncytial virus disease. *Am J Respir Crit Care Med* 1999;160:1263-8.
37. Bont L, Heijnen CJ, Kavelaars A, van Aalderen WM, Brus F, Draaisma JT, et al. Peripheral blood cytokine responses and disease severity in respiratory syncytial virus bronchiolitis. *Eur Respir J* 1999;14:144-9.
38. Renzi PM, Turgeon JP, Marcotte JE, Drblik SP, Berube D, Gagnon MF, et al. Reduced interferon-gamma production in infants with bronchiolitis and asthma. *Am J Respir Crit Care Med* 1999;159:1417-22.
39. Legg JP, Hussain IR, Warner JA, Johnston SL, Warner JO. Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. *Am J Respir Crit Care Med* 2003;168:633-9.
40. Bennett BL, Garofalo RP, Cron SG, Hosakote YM, Atmar RL, Macias CG, et al. Immunopathogenesis of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2007;195:1532-40.
41. Garcia C, Soriano-Fallas A, Lozano J, Leos N, Gomez AM, Ramilo O, et al. Decreased innate immune cytokine responses correlate with disease severity in children with respiratory syncytial virus and human rhinovirus bronchiolitis. *Pediatr Infect Dis J* 2012;31:86-9.
42. Bont L, Heijnen CJ, Kavelaars A, van Aalderen WM, Brus F, Draaisma JM, et al. Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J Infect Dis* 2001;184:355-8.
43. Lee YM, Miyahara N, Takeda K, Prpich J, Oh A, Balhorn A, et al. IFN-gamma production during initial infection determines the outcome of reinfection with respiratory syncytial virus. *Am J Respir Crit Care Med* 2008;177:208-18.
44. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005;201:937-47.
45. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006;12:1023-6.
46. Bullens DM, Decraene A, Dilissen E, Meyts I, De Boeck K, Dupont LJ, et al. Type III IFN-lambda mRNA expression in sputum of adult and school-aged asthmatics. *Clin Exp Allergy* 2008;38:1459-67.
47. Miller EK, Hernandez JZ, Wimmenauer V, Shepherd BE, Hijano D, Libster R, et al. A mechanistic role for type III IFN-lambda in asthma exacerbations mediated by human rhinoviruses. *Am J Respir Crit Care Med* 2012;185:508-16.
48. Bont L, Steijn M, Van Aalderen WM, Brus F, Th Draaisma JM, Van Diemen-Steenvoorde RA, et al. Seasonality of long term wheezing following respiratory syncytial virus lower respiratory tract infection. *Thorax* 2004;59:512-6.
49. Dakhama A, Lee YM, Ohnishi H, Jing X, Balhorn A, Takeda K, et al. Virus-specific IgE enhances airway responsiveness on reinfection with respiratory syncytial virus in newborn mice. *J Allergy Clin Immunol* 2009;123:138-45.e5.
50. Kotaniemi-Syrjänen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;111:66-71.
51. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol* 2015;136:81-6.e4.
52. Heymann PW, Carper HT, Murphy DD, Platts-Mills TA, Patrie J, McLaughlin AP, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. *J Allergy Clin Immunol* 2004;114:239-47.
53. Simoes EA, Carbonell-Estrany X, Rieger CH, Mitchell I, Fredrick L, Groothuis JR. The effect of respiratory syncytial virus on subsequent recurrent wheezing in atopic and nonatopic children. *J Allergy Clin Immunol* 2010;126:256-62.
54. Yoshihara S, Kusuda S, Mochizuki H, Okada K, Nishima S, Simoes EA. Effect of palivizumab prophylaxis on subsequent recurrent wheezing in preterm infants. *Pediatrics* 2013;132:811-8.
55. Kim CK, Choi J, Kim HB, Callaway Z, Shin BM, Kim JT, et al. A randomized intervention of montelukast for post-bronchiolitis: effect on eosinophil degranulation. *J Pediatr* 2010;156:749-54.