

Symptomatic Viral Infection is Associated with Impaired Response to Treatment in Children with Acute Asthma

Kristina Rueter, MD, Joeline Bizzantino, PhD, Andrew C. Martin, MD, Guicheng Zhang, PhD,
Catherine M. Hayden, PhD, Gary C. Geelhoed, MD, Jack Goldblatt, MD,
Ingrid A. Laing, PhD, and Peter N. Le Souëf, MD

Objective To examine the influence of viral respiratory infection (VRI) on treatment response in acute asthma in children.

Study design A total of 218 children (mean age, 6.6 years) with acute asthma were recruited. Symptoms were recorded, an asthma severity score was determined, and whenever possible, a per-nasal aspirate was obtained for detection of viruses. Each child's response to inhaled β_2 -agonists was assessed after 6, 12, and 24 hours.

Results The 168 children with VRI symptoms received more treatment with inhaled β_2 -agonists after 6 hours ($P = .010$), 12 hours ($P = .002$), and 24 hours ($P = .0005$) compared with the 50 children without such symptoms. Asthma severity did not differ between the 2 groups. A per-nasal aspirate was obtained from 77% of the children. The most frequently identified virus was rhinovirus (61.4%). Among children with symptoms of a VRI, those with rhinovirus had an impaired response to β_2 -agonists at 6 hours ($P = .032$).

Conclusion Children with acute asthma and symptoms of VRI respond less effectively to β_2 -agonists after 6, 12, or 24 hours and thus may benefit from more intense therapy and monitoring. (*J Pediatr* 2011;■:■-■).

Asthma is the most common admission diagnosis for children in many developed countries, and exacerbations of asthma symptoms are associated with significant costs for healthcare systems.^{1,2} Epidemiologic studies have demonstrated that 60%-85% of acute asthma exacerbations are associated with a viral respiratory infection (VRI).^{3,4} Approximately two-thirds of these infections are due to rhinovirus (RV), but other respiratory viruses have been identified as well.⁵ Respiratory viruses have been associated with airway inflammation and narrowing, with resulting reduced lung function in children.^{3,7} Respiratory viruses also may act synergistically with other factors associated with asthma, particularly sensitization and exposure to allergens.⁶ However, whereas the mechanisms of virus-induced asthma exacerbations have been studied intensively using in vitro and in vivo experimental models,⁷ the possibility that viruses could influence treatment response during an acute exacerbation has not yet been defined.

For the present study, we selected the presence of VRI symptoms as a marker of viral infection in the first instance, because this is a presenting phenotype that represents a clinically practical way to identify children who may be infected with a virus and may require more intense treatment to stabilize their asthma symptoms. We subsequently conducted virus detection to determine specific viral effects on treatment outcome. Knowledge of response to β_2 -agonists is likely to be particularly relevant, given that these agents are the most effective and commonly prescribed treatment for asthma exacerbations in children and adults.

To the best of our knowledge, no study to date has examined the influence of clinical symptoms of VRI or different viral infections on treatment response during acute asthma exacerbations. This information is potentially important in establishing more effective treatment for acute asthma. Consequently, the present study examined the following questions: (1) Do children with acute asthma with or without VRI symptoms differ in terms of the severity of attacks, oxygen saturation (SaO_2), total immunoglobulin E (IgE) level, or specific IgE (sIgE) level? (2) Does response to β_2 -agonists differ between children

ED	Emergency department
IgE	Immunoglobulin E
INF	Interferon
PCR	Polymerase chain reaction
PNA	Per-nasal aspirate
RSV	Respiratory syncytial virus
RV	Rhinovirus
SaO_2	Oxygen saturation
sIgE	Specific IgE
VRI	Viral respiratory infection

From the Princess Margaret Hospital for Children (K.R., A.M., G.G., P.L.); School of Paediatrics and Child Health, University of Western Australia (K.R., J.B., A.M., G.Z., C.H., G.G., J.G., I.L., P.L.); and Telethon Institute for Child Research, Centre for Child Health Research (J.B., I.L.), Perth, Australia

Supported by grants from the National Health and Medical Research Council of Australia, Asthma Foundation of Western Australia, and West Australian Institute of Medical Research. The authors declare no conflicts of interest.

0022-3476/\$ - see front matter. Copyright © 2011 Mosby Inc. All rights reserved. 10.1016/j.jpeds.2011.06.025

with and those without VRI symptoms during an acute asthma exacerbation? (3) Is there an association between specific viruses isolated from children with acute asthma and response to treatment with β_2 -agonists?

These questions correspond with the general hypotheses that the presence of a symptomatic viral respiratory illness in children with acute asthma is associated with more severe episodes (as reflected by severity score on presentation) and that these children will be less responsive to treatment (as reflected by administered doses of bronchodilator therapy).

Methods

The study was designed as a prospective observational study and was approved by the Ethics Committee at Princess Margaret Hospital for Children, Perth, Australia. Patients with an acute asthma exacerbation were recruited after presentation at the hospital's Emergency Department (ED), as described previously.⁸ Before participation, parental written informed consent was obtained for each child.

Patients with any other underlying chronic lung condition were excluded from the analysis. Based on symptoms reported by parents using a standardized questionnaire and clinical examination by an ED doctor, the children were dichotomized to a group with symptoms consistent with VRI and a group without these symptoms (Figure 1). VRI was defined as a combination of symptoms including runny nose, sore throat, and cough. Study personnel played no role in clinical diagnosis, to avoid introducing any bias.

The severity of an acute asthma attack at presentation was determined using a previously modified National Institutes of Health scoring system,⁹ with a possible score of 5–15. SaO_2 was recorded in every patient. Atopy was characterized by total IgE and sIgE levels.

All patients received the same initial therapy, including salbutamol 100 μg per actuation via spacer every 20 minutes for the first hour, oral prednisolone 1 mg/kg, and supplemental oxygen if SaO_2 was $\leq 94\%$.⁸ According to our ED's treatment protocol, administration of salbutamol implied 6 actuations in children aged <6 years and 12 actuations in children aged >6 years. β_2 -agonist doses were administered by trained nursing staff. Management decisions regarding subsequent administrations of β_2 -agonists were based on clinical grounds and recorded by the ED physicians without any input from study personnel. The treatment response of subgroups was based on the total frequency of β_2 -agonist administration after 6, 12, and 24 hours. All participants remained in the ED for at least the first 24 hours.

Samples

In all children, peripheral blood samples were obtained directly after initiation of treatment. Plasma was stored at -80°C . A per-nasal aspirate (PNA) was collected at presentation.

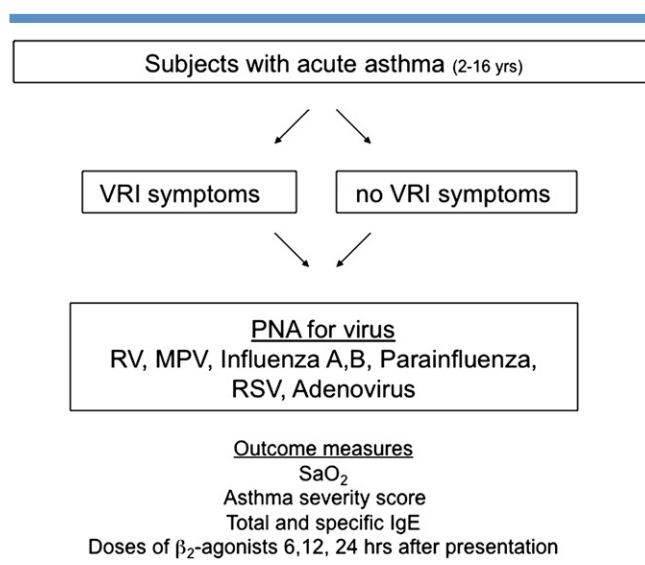


Figure 1. After recruitment, children with asthma were divided into those with VRI symptoms and those without VRI symptoms before undergoing PNA for viruses and analysis of outcome measures. MPV, human metapneumovirus.

Plasma Assays

Plasma levels of total IgE and sIgE to *Dermatophagoides pteronyssinus* (house dust mite) and cat pelt were measured using the total IgE Cap-FEIA and radioallergosorbent test IgE Cap-FEIA tests (Pharmacia, Uppsala, Sweden).

Viral Detection

PNAs were analyzed by local diagnostic pathology laboratories. RV and human metapneumovirus were detected by RNA extraction and reverse-transcription polymerase chain reaction (PCR).^{10,11} Respiratory syncytial virus (RSV), adenovirus, and influenza and parainfluenza viruses were identified either by direct immunofluorescence using virus-specific monoclonal antibodies or by isolation in cell culture (LLC-MK₂, A549, and MRC₅ cell lines) and identification by indirect immunofluorescence (Meridian Bioscience, Cincinnati, Ohio; Dako Diagnostics, Glostrup, Denmark).

Statistical Analysis

Data were analyzed using SPSS 15.0 for Windows (SPSS Inc, Chicago, Illinois). The independent-sample *t*-test was used for normally distributed variables, and the nonparametric Mann-Whitney *U* test was used for nonnormally distributed parameters. Significant results of parametric analyses were adjusted for age and sex by linear regression. Significance was set at $P < .05$.

Results

A total of 218 children (mean age, 6.6 ± 3.5 years; 138 males) with an acute asthma attack were recruited. Of these 218 children, 168 had VRI symptoms. The children's mean

SaO₂ was 93.7% $\pm$ 3.4%, and mean asthma severity score was 9.9 $\pm$ 2.3.

Differences in Acute Asthma Exacerbations in Subjects with and without VRI Symptoms

There was no significant difference in SaO₂, asthma severity score, total IgE, or sIgE levels for cat or house dust mite between children with or without VRI symptoms. In addition, the time since onset of acute asthma symptoms and presentation to the ED did not differ significantly between the 2 groups. The children with VRI symptoms tended to report to the ED sooner, however (Table I).

Treatment Response to β_2 -Agonists

Compared with children without VRI symptoms, those with VRI symptoms required significantly more doses of β_2 -agonists at 6 hours ($P = .010$), 12 hours ($P = .002$), and 24 hours ($P = .0005$) after presentation to the ED (Table I and Figure 2). These associations remained significant after correction for age and sex. Atopic status was not related to treatment response.

Role of Respiratory Viruses in Treatment Response

We examined the possibility that different respiratory viruses could exert different effects on treatment response times in children with preceding VRI symptoms. PNAs were taken in 77% (168/218). A virus was isolated in 64% (107/168) and of those, 61.4% were positive for RV (94/153), 5.6% for RSV (9/162), 3.7% for human metapneumovirus (3/82), 0.6% for influenza A and parainfluenza (1/160). None of the PNAs were positive for influenza B (0/160) or adenovirus (0/158). One child had a dual infection with RV and RSV. Out of all virus-positive PNAs, 88% were RV-positive

(94/107). As expected, RV was detected more frequently in those children with symptoms of VRI (Table I).

Impact of Virus Detection on Response to β_2 -Agonists in Patients with VRI Symptoms. Children with VRI symptoms were divided into those positive and those negative for viruses detected in the PNA specimen (Table II). Response to inhaled β_2 -agonists was not significantly different between these 2 groups at 6, 12, and 24 hours after presentation.

Virus-specific subanalysis could be done only for RV because of the low number of non-RV isolates. There was no significant difference in treatment response between subjects with RV and those without RV.

Compared with subjects with any other respiratory viruses detected, those positive for RV required significantly higher doses of β_2 -agonists at 6 hours after presentation; significance remained after correction for age and sex. A similar trend was seen at 12 hours after presentation, but not at 24 hours.

Impact of Virus Detection on Response to β_2 -Agonists in Patients with and without VRI Symptoms.

Patients with VRI symptoms, regardless of positive or negative virus detection status, required more doses of salbutamol than those without VRI symptoms (Table II). Patients with VRI symptoms without RV still required a greater number of doses of β_2 -agonists than those without VRI symptoms. This relationship was significant even after adjustment for age and sex at 12 and 24 hours after presentation, and a similar trend that did not reach statistical significance was seen at 6 hours after presentation.

Finally, we compared treatment response in children with VRI symptoms who were negative for viruses ($n = 42$) and

Table I. Comparison of children with acute asthma with and without VRI symptoms

	VRI symptoms (n = 168)	No VRI symptoms (n = 50)	P value
Days of asthma symptoms before presentation, mean (95% CI)*	1.92 (1.51-2.34)	3.67 (-0.25 to 7.59)	.088
% SaO ₂ , median (25th-75th percentile)	94.0 (92.0-96.0)	95.0 (91.0-96.0)	NS
Asthma severity score, mean (95% CI)†	10.0 (9.6-10.3)	9.9 (9.2-10.6)	NS
Total IgE, kU/L, GM (95% CI)‡	229.7 (185.4-284.6)	265.7 (179.2-394.0)	NS
Cat sIgE, kU/L, mean $\pm$ SD§	4.3 (10.6)	11.8 (35.5)	NS
House dust mite sIgE, kU/L, mean $\pm$ SD§	42.0 (49.5)	56.4 (68.3)	NS
β_2 -agonist dose, GM (95% CI)			
At 6 hours¶	6.9 (6.5-7.3)	5.9 (5.3-6.5)	.010
At 12 hours¶	10.4 (9.7-11.0)	8.4 (7.5-9.5)	.002
At 24 hours¶	15.1 (14.1-16.1)	11.5 (9.9-13.4)	.0005
Virus detected, n/n tested (%)			
RV	81/123 (65.9)	13/30 (43.3)	.023
RSV	9/131 (6.9)	0/31 (0)	NS
Human metapneumovirus	2/62 (3.2)	1/20 (5.0)	NS
Influenza A	0/130 (0)	1/30 (3.3)	NS
Influenza B	0/130 (0)	0/30 (0)	NS
Parainfluenza	1/130 (0.8)	0/130 (0)	NS
Adenovirus	0/128 (0)	0/30 (0)	NS

GM, geometric mean; NS, not significant at $P < .05$.

* $n = 113$ for VRI symptoms, $n = 27$ for no VRI symptoms.

† $n = 49$ for no VRI symptoms.

‡ $n = 130$ for VRI symptoms, $n = 36$ for no VRI symptoms.

§ $n = 131$ for VRI symptoms, $n = 36$ for no VRI symptoms.

¶ $n = 167$ for VRI symptoms, $n = 50$ for no VRI symptoms.

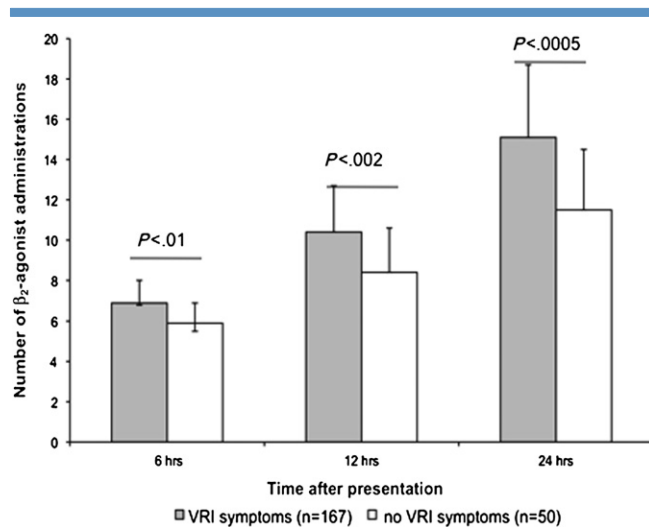


Figure 2. Administration of β_2 -agonists (geometric mean with 95% CI) in children with VRI symptoms and those without VRI symptoms.

children without VRI symptoms who were positive for a virus ($n = 13$). There was no significant difference between these 2 groups.

Discussion

This study demonstrates that the response to β_2 -agonists in children with acute asthma depends on the presence of clinical symptoms of VRI. Among children presenting with acute

asthma to our ED, those with VRI symptoms required higher doses of salbutamol compared with those without these symptoms. This finding was independent of attack severity, given that SaO_2 and acute asthma severity score were not influenced by VRI symptoms. Moreover, in children in whom a virus was isolated, RV had a greater effect on treatment response than other viruses. These data support the likelihood that RV has a stronger and unique relationship with acute asthma in children compared with other viruses.

An association between “colds” and exacerbation of asthma has long been recognized.¹² In our cohort, 77% of the children presenting to the ED with an acute asthma exacerbation had clinical symptoms of a VRI. Despite the tendency for children with VRI symptoms to present for medical attention sooner, these children required significantly higher doses of short-acting β_2 -agonists for at least 24 hours than the children without these symptoms.

There was no difference in the severity of initial acute asthma presentation or IgE levels^{13–15} between the children with and those without VRI symptoms. This suggests that identifying VRI symptoms at the initial assessment is of potential clinical importance, given that those children with such symptoms may benefit from more intense therapy and monitoring.

We also examined whether the isolation of specific viruses in children with acute asthma might influence the response to treatment with β_2 -agonists. Previous studies showed varying results with respect to frequency of viral isolation.^{16–18}

In the present study, viruses in PNA were detected in 64% of children presenting with acute asthma to the ED at Princess Margaret Hospital for Children, the sole tertiary pediatric referral center in Western Australia. This rate is low compared with rates reported in previous studies.^{3,4}

Table II. Influence of viral detection in PNA and VRI symptoms on treatment response to β_2 -agonists in children with acute asthma exacerbations

Groups	n	Doses of β_2 -agonists, geometric mean (95% CI)		
		6 hours	12 hours	24 hours
VRI symptoms and PNA positive for virus versus VRI symptoms and PNA negative for virus	n = 92 n = 43	7 (6.4-7.5) 7 (6.2-7.4) <i>P</i> = .768	10 (9.4-11.1) 11 (9.4-11.8) <i>P</i> = .712	15 (13.5-16.4) 15 (13.6-17.5) <i>P</i> = .626
VRI symptoms and PNA positive for RV versus VRI symptoms and PNA negative for RV	n = 81 n = 42	7 (6.5-7.8) 7 (6.0-7.3) <i>P</i> = .296	10 (9.6-11.5) 10 (9.2-11.6) <i>P</i> = .809	15 (13.7-17.0) 15 (13.4-17.3) <i>P</i> = .965
VRI symptoms and PNA positive for RV versus VRI symptoms and PNA positive for any virus other than RV	n = 80 n = 12	7 (6.6-7.8) 5 (4.7-6.1) <i>P</i> = .015 <i>*P</i> = .032	11 (9.6-11.6) 8 (6.9-9.9) <i>P</i> = .059	15 (13.7-17.1) 12 (9.8-14.9) <i>P</i> = .106
VRI symptoms and PNA positive for virus versus No VRI symptoms	n = 92 n = 50	7 (6.4-7.5) 6 (5.3-6.5) <i>P</i> = .016 <i>*P</i> = .016	10 (9.4-11.1) 8 (7.5-9.5) <i>P</i> = .009 <i>*P</i> = .009	15 (13.5-16.4) 12 (9.9-13.4) <i>P</i> = .004 <i>*P</i> = .004
VRI symptoms and PNA negative for virus versus No VRI symptoms	n = 43 n = 50	7 (6.2-7.4) 6 (5.3-6.5) <i>P</i> = .048 <i>*P</i> = .095	11 (9.4-11.8) 8 (7.5-9.5) <i>P</i> = .009 <i>*P</i> = .020	15 (13.6-17.5) 12 (9.9-13.4) <i>P</i> = .004 <i>*P</i> = .009
VRI symptoms and PNA negative for RV versus No VRI symptoms	n = 42 n = 50	7 (6.0-7.3) 6 (5.3-6.5) <i>P</i> = .088	10 (9.2-11.6) 8 (7.5-9.5) <i>P</i> = .017 <i>*P</i> = .035	15 (13.4-17.3) 12 (9.9-13.4) <i>P</i> = .007 <i>*P</i> = .015

Significant *P* values are in bold.

**P* value after adjustment for age and sex by linear regression.

Consistent with previous reports, the predominant virus detected was RV. RV was detected in 88% of virus-positive PNAs, a higher rate than reported in previous studies. This difference might be related to detection methods, geographic and environmental factors, period and season of recruitment, and patient age and sex.¹⁹

In our analysis, treatment response to bronchodilators was significantly affected by clinical symptoms of a VRI, but association between treatment response and viral isolation was more complex. The lack of a relationship between VRI symptoms and severity was not unexpected, given the expectation that the severity of symptoms that would cause parents to bring a child to the hospital would depend on the child surpassing a particular level of wheezing and breathlessness independent of other factors.

In addition, we examined whether other respiratory viruses influenced treatment response to salbutamol. Children positive for RV required significantly higher doses of β_2 -agonists at 6 hours after presentation compared with children positive for other respiratory viruses tested. A similar trend was seen at 12 hours. Although the number of children with other viruses was small, these data support the emerging view that RV has a stronger relationship with asthma compared with other respiratory viruses. The strength of this relationship is evident early in life; infection with RV during the first 3 years of life is much more likely to predict the diagnosis of asthma at age 6 years compared with any other virus or epidemiologic variable.²⁰

The presence of VRI symptoms in the absence of laboratory detection of a respiratory virus does not necessarily mean the absence of a virus. Recent studies using extended, custom-designed PCR methodologies found that some RV strains were not detected by existing techniques.²¹⁻²³ Thus, some of the children in our study with VRI symptoms might have had an RV infection that could only be detected using this new approach. This might explain the significant difference in bronchodilator doses between children with VRI symptoms who had a virus-negative PNA and those with no VRI symptoms, and might explain why β_2 -agonist doses were not significantly different in children positive and negative for RV. It also could explain why children with RV infection had a worse response to β_2 -agonists than those with other viruses, but not worse than children with no viral infection, because a proportion of those negative for viruses might have had an RV infection. Further studies of the role of RV in acute asthma should be undertaken when standardized techniques incorporating extended PCR detection for RV become available.

The mechanisms of RV-induced asthma exacerbations remain poorly understood.²⁴ Previous studies have suggested that adults with asthma have increased susceptibility to naturally occurring RV infections because of their deficient innate immune responses.²⁵ In asthma, increased viral replication in bronchial epithelial cells occurs due to deficiencies in the induction of apoptosis and production of interferon (INF)- β and INF- λ 1-3. However, further studies are needed to identify whether those deficiencies play a role in children as well. Furthermore, it would be interesting to know if those defi-

ciencies are also observed in infections with pathogens other than RV or in children with clinical symptoms of a VRI. If so, treatment with INF- β and/or INF- λ might be beneficial.²⁶

Whether there is a window of opportunity between the occurrence of VRI symptoms and the development of acute asthma exacerbation during which antiviral therapy might be effective is unknown.²⁷ Immunization options for influenza or RV remain unsatisfactory.^{28,29} Our finding that clinical symptoms of VRI predict treatment response to β_2 -agonists in acute asthma supports the need for future studies of treatment options, including early specific antiviral strategies and targeting of anti-inflammatory pathways.

The present study has several potential limitations. It was not possible to obtain a PNA for virus detection in all recruited children, because those without VRI symptoms had little or no nasal mucus to aspirate. In addition, because of our institution's laboratory protocols, not all respiratory viruses were tested in every patient, and not all of the methods for virus detection were PCR-based. Whereas RSV detection by monoclonal antibody or isolation in cell culture has a sensitivity of 95%, detection of influenza and adenovirus (80% sensitivity) might have been slightly higher with PCR. However, routine diagnostic methodology encompasses the quickest and most appropriate isolation for clinical practice. Because there were limited isolates of respiratory viruses other than RV, the study have been underpowered to detect a relationship between the latter viruses and the severity of asthma exacerbation.

The frequency of administration of β_2 -agonists served as our primary measure of clinical response. This approach is subject to ED physician assessment, which might vary among patients but nonetheless provides an unbiased view of the actual treatment received by children presenting with an acute asthma attack. More objective markers, such as spirometry parameters, would have been difficult to assess reliably in most of the patients due to the severity of the asthma attack or young age. Furthermore, an average of more than 24 hours passed before children were well enough to perform lung function tests; thus, these data were not suitable for analyses with treatment response in the first 24 hours since ED presentation. Steroid therapy was not an outcome measure of this study, given that the ED protocol resulted in all children receiving oral steroids.

The timing of other treatment administered before presentation to the ED and the duration of preceding infection are potential study cofounders. Furthermore, we cannot discount the possibility that allergen exposure might have played a role as well, but clearly this would have been very difficult to study objectively.^{6,19,26}

In conclusion, this study of children with acute asthma demonstrates that those with symptoms of a VRI required more doses of β_2 -agonists at 6, 12, and 24 hours after presentation to the ED. Therefore, identifying VRI symptoms at the initial assessment would be of potential clinical importance, given that children with such symptoms may benefit from more intensive therapy and monitoring. Clinical severity, SaO₂, and total IgE and sIgE levels did not differ between

the children with VRI symptoms and those without these symptoms. This indicates that the differing response to treatment in children with and without VRI symptoms was not due to differences in acute asthma or allergy phenotypes at presentation. RV was by far the most frequently isolated virus, and children infected with RV were more likely to require more doses of β_2 -agonists. Further investigation is needed to compare mechanisms of pathogenesis of RV and other respiratory viruses and to investigate their influence on treatment outcome in greater detail. ■

Submitted for publication Jan 22, 2011; last revision received Apr 21, 2011; accepted Jun 22, 2011.

Reprint requests: Kristina Rueter, MD, Princess Margaret Hospital, Roberts Road, Subiaco, WA 6008, Australia. E-mail: kristina.rueter@health.wa.gov.au

References

1. Lane S, Molina J, Plusa T. An international observational prospective study to determine the cost of asthma exacerbations (COAX). *Respir Med* 2006;100:434-50.
2. Skrepnek GH, Skrepnek SV. Epidemiology, clinical and economic burden, and natural history of chronic obstructive pulmonary disease and asthma. *Am J Manag Care* 2004;10:S129-38.
3. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9- to 11-year-old children. *BMJ* 1995;310:1225-9.
4. Heymann PW, Platts-Mills TA, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. *Pediatr Infect Dis J* 2005;24:S217-22.
5. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 2002;359:831-4.
6. Murray CS, Poletti G, Kebabdz T, Morris J, Woodcock A, Johnston SL, et al. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006;61:376-82.
7. Papadopoulos NG, Xepapadaki P, Mallia P, Brusselle G, Watelet JB, Xatzipsalti M, et al. Mechanisms of virus-induced asthma exacerbations: state of the art. A GA2LEN and InterAirways document. *Allergy* 2007;62:457-70.
8. Martin AC, Laing IA, Khoo SK, Zhang G, Rueter K, Teoh L, et al. Acute asthma in children: relationships among CD14 and CC16 genotypes, plasma levels, and severity. *Am J Respir Crit Care Med* 2006;173:617-22.
9. Qureshi F, Pestian J, Davis P, Zaritsky A. Effect of nebulized ipratropium on the hospitalization rates of children with asthma. *N Engl J Med* 1998;339:1030-5.
10. Myint S, Johnston S, Sanderson G, Simpson H. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. *Mol Cell Probes* 1994;8:357-64.
11. Ireland DC, Kent J, Nicholson KG. Improved detection of rhinoviruses in nasal and throat swabs by seminested RT-PCR. *J Med Virol* 1993;40:96-101.
12. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996;154:654-60.
13. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial. *Lancet* 2003;362:1433-8.
14. Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, et al. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 1997;337:1405-11.
15. Doull IJ, Lampe FC, Smith S, Schreiber J, Freezer NJ, Holgate ST. Effect of inhaled corticosteroids on episodes of wheezing associated with viral infection in school age children: randomised double-blind placebo-controlled trial. *BMJ* 1997;315:858-62.
16. Mallia P, Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest* 2006;130:1203-10.
17. 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.
18. Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, et al. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med* 2005;172:433-9.
19. Johnston NW, Sears MR. Asthma exacerbations, 1: epidemiology. *Thorax* 2006;61:722-8.
20. 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.
21. Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, et al. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS ONE* 2007;2:e966.
22. Miller EK, Edwards KM, Weinberg GA, Iwane MK, Griffin MR, Hall CB, et al. A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immunol* 2009;123:98-104.
23. Leung TF, To MY, Yeung AC, Wong YS, Wong GW, Chan PK. Multiplex molecular detection of respiratory pathogens in children with asthma exacerbation. *Chest* 2010;137:348-54.
24. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 2005;116:267-73.
25. 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.
26. Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. *Proc Am Thorac Soc* 2007;4:267-70.
27. Velissariou IM, Papadopoulos NG. The role of respiratory viruses in the pathogenesis of pediatric asthma. *Pediatr Ann* 2006;35:637-42.
28. Bueving HJ, van der Wouden JC. What is the role of virus vaccination in patients with asthma? *Curr Allergy Asthma Rep* 2007;7:72-6.
29. Papadopoulos NG, Papi A, Psarras S, Johnston SL. Mechanisms of rhinovirus-induced asthma. *Paediatr Respir Rev* 2004;5:255-60.