

Persistence of rhinovirus RNA after asthma exacerbation in children

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Summary

Background Rhinoviruses (RVs) are believed to cause most asthma exacerbations but their role in the severity of acute asthma and subsequent recovery of airway function is not defined. The importance of atopy in virus-host interactions is also not clear.

Objective We postulated that RV infection and atopic skin prick responses influence the severity of asthma exacerbations as measured by peak expiratory flow (PEF).

Methods Patients aged 4–12 years admitted with acute severe asthma to a hospital emergency room (ER) were recruited. PEF measurements were obtained and nasal aspirates (NA) were taken. Atopy was diagnosed by skin prick responses to allergen and the presence of RV RNA and respiratory syncytial virus (RSV) RNA in NAs was detected using validated PCR assays. Patients were restudied after 6 weeks and after 6 months.

Results Fifty children with acute asthma (mean age $\pm$ SD, 7.4 ± 2.7) were enrolled; atopy was present in 37 (74%). RV RNA was detected in 41 (82%) and RSV RNA in six (12%) subjects. After 6 weeks 41 patients were restudied and RV RNA was again detected in 18 (44%). RV RNA was detected after 6 months in four of 16 patients restudied (25%; $P = 0.008$ vs. ER) and in two of nine children from a control group with stable asthma (22%; $P = 0.009$ vs. ER). Overall PEF measurements were reduced in asthmatics admitted to ER (% predicted, $63.4 \pm 16.4\%$) but did not differ between patients with RV RNA, RSV RNA or neither virus present. In subjects with RV RNA detectable in ER and after 6 weeks, measurements of PEF in ER were significantly lower than in patients in whom RV RNA was present in ER but absent after 6 weeks ($P = 0.009$). Regression analysis linked persistence of RV RNA, but not skin prick responses to allergen, to severity of PEF reductions in ER.

Conclusion RV RNA was detectable in >40% of asthmatic children 6 weeks after an acute exacerbation. Asthma exacerbations were more severe in patients with persistence of RV RNA suggesting that the severity of acute asthma may be linked to prolonged and possibly more severe RV infections.

Keywords asthma, exacerbation, PEF, rhinovirus

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Introduction

Rhinovirus (RV) is the predominant common cold virus in children and can be identified by culture methods in up to one third of wheezing attacks [1]. Detection has been improved by molecular methods, particularly RT-PCR; these assays have demonstrated RV RNA associated with mild exacerbations of asthma in up to 80% of children [2] and more than 30% of severe attacks in adults [3]. Respiratory syncytial virus (RSV) has also been implicated in wheezing illness in children and a number of studies have established that its prevalence is second only to RV [2, 4, 5].

Acute asthma results in significant morbidity and mortality [6, 7]. Although exacerbations are generally managed in a uniform fashion, there are considerable inter-patient differences pertaining to precipitating factors, pattern of onset, severity of airflow obstruction, duration of the attack and response to treatment. Studies of near-fatal asthma have examined elements predictive of acute asthma severity and several host characteristics of presumed importance have been identified [6–8]. These studies did not indicate whether infectious agents such as viruses are the cause of more severe attacks than exacerbations linked to allergen or whether a particular group of viruses may be associated with greater severity. However, there is some ancillary evidence that viral infections may influence the severity of airway obstruction. Firstly, studies of asthma death have demonstrated two distinct patterns of pathology and it was proposed that viral infections might be linked to asthma death characterized by

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an influx of neutrophils into the airways [9]. Secondly, it was recently shown that high NO₂ exposure linked to RV infection enhances the severity of exacerbations compared with other causes [10]. Finally, there is recent evidence that RV is the cause of more severe acute bronchiolitis in infants compared with other respiratory pathogens [11]. Thus, although a key role is postulated for RV as a cause of asthma exacerbations, it is not clear whether the virus determines severity compared with other provocants of acute attacks.

A significant number of children may harbour RV when asymptomatic [2, 4]. As yet it is not known if detection of RV in this context reflects a long-term carrier state or whether it is the result of ongoing but delayed resolution of prior infection. It is known that RT-PCR assays can remain positive in 50% of volunteers for up to 14 days after experimental RV infection [12] suggesting that new infections may persist for a prolonged period in the absence of cold symptoms. Our previous studies of community-acquired RV infections in adult acute asthma have suggested that RV is eliminated by 6 weeks as judged by RT-PCR assays [3]. However, to date no studies have evaluated persistence of RV in children and determined whether the length of this period is linked to severity of the infection or other host characteristics.

This study examined the prevalence of RV and RSV in children with acute asthma in the setting of an ER. Moreover, we postulated that RV-linked exacerbations and atopy are factors associated with greater severity and used PCR assays, pulmonary function measurements and skin prick responses to allergen to examine their contribution to severity.

Methods

Study populations

In the period of January 1998–July 1999, 50 asthmatic patients (from a total of approximately 280 acute asthmatics seen in ER) were recruited from the ER of Tygerberg Hospital, Red Cross Children's Hospital and Constantiaberg Clinic, Cape Town, SA. Children were aged between 4 and 12 years and had a diagnosis of asthma documented in their hospital or clinic records. All but two children had a history of at least one previous visit to ER for acute asthma. Symptoms of an upper respiratory tract infection (URTI) were documented as previously reported [2]. Acute lower respiratory symptoms and their duration were documented (chest tightness, wheezing, breathlessness, night asthma) but assessment of symptom severity was not attempted because initial evaluation suggested that accurate estimation of severity of acute symptoms in children was not feasible. Children had wheezing detected by auscultation in ER, were managed by β -agonist nebulization and given oral corticosteroids (OCS) for 3–21 (mean 5.3) days [13]. Patients who had used OCS in a 6-week period prior to admission were excluded. Demographic information is shown in Table 1.

Patients were restudied after 6 weeks ($n = 41$) and after 6 months ($n = 16$). Although all patients were asked to return for study after 6 weeks, nine did not return because of personal or family household reasons. Patients were selected from the 6 week group for repeat study after 6 months using a

Table 1. Baseline demographic data of 50 asthmatic children admitted to an emergency room (ER)

	RV not detected ($n = 9$)	RV detected ($n = 41$)
Age (mean $\pm$ SD)	7.3 $\pm$ 3.0	7.4 $\pm$ 2.7
Duration of asthma (years)	5.3 $\pm$ 3.4	5.0 $\pm$ 2.6
Symptoms URTI	9	40
Time to ER* (h)	5.6 $\pm$ 3.9	7.9 $\pm$ 6.7
Atopy (skin testing)	9	28
History of hayfever	6	28
Medication†	ICS = 8; T = 1	ICS = 32; T = 7
PEF (% predicted)	63.4 $\pm$ 16.5	62.1 $\pm$ 16.7
PEF after β_2 -agonist nebulization	69.8 $\pm$ 20.3	67.9 $\pm$ 16.1

PEF, peak expiratory flow measured in ER; RV, rhinovirus; URTI, upper respiratory tract infection.

*Time from onset of worsening of asthma to ER attendance.

†ICS, inhaled corticosteroids; T, theophylline, all patients had used β_2 -agonists

randomized schedule (a subgroup was chosen because study limitations precluded evaluation of the entire group). At the time of the 6 month review a control group ($n = 9$) of asthmatic children were also recruited from outpatient clinics; they had clinically stable asthma for at least 3 months without any hospital admission or use of OCS. The study was approved by the Human Research Ethics Committees of Stellenbosch and Cape Town Universities, Cape Town, and all patients' parents signed written informed consent.

Specimen collection

Nasal aspirates (NA) were obtained as previously described [14]. Briefly, nasal mucus was obtained with a suction pump after administration of histamine (8 mg/mL) as a fine spray into each nostril. Histamine spray ensures that an adequate nasal sample can be obtained from children without copious nasal secretions [2]. The NA (~ 1 mL) was mixed with 3 mL of viral transport medium (10% Hanks balanced salt solution, 0.015 M sodium bicarbonate, 40 μ g/mL Ampicillin, 200 μ g/mL Amphotericin B, 0.5% bovine serum albumin), transported on ice, aliquots made and frozen at -80°C until further analysis. Initial studies indicated that very few patients were able to produce a sputum sample and this aspect was not pursued in the study.

Virus detection

RV was detected by RT-PCR as we have described [3, 14, 15]. Briefly, 150 μ L of NA sample was subjected to RNA extraction using a phenol-chloroform reagent (Tri Reagent, Sigma, Melbourne, Australia). Dried nucleic acid pellets were resuspended in 5 μ L DEPC-water and after RT [BRL Superscript Reverse Transcriptase, Gibco (Invitrogen), Melbourne, Australia] PCR was performed as follows: 30 cycles of 45 s at 94°C , 45 s at 53°C and 75 s at 72°C , followed by a 10 min incubation at 72°C . Samples were assayed in a total volume of 50 μ L, each containing 20 mM of dCTP, dGTP, dATP and dTTP, 50 pmol of each primer (OL-26 and OL-27), 5 units of Taq DNA Polymerase (Gibco) and 50 mM MgCl₂. The product (10 μ L) was electrophoresed on 0.7% agarose/TAE gels and a positive result indicated by a band at the

380 bp position. The specificity of the assay has been demonstrated [14–16]. All samples were analysed in a blinded fashion and in duplicate. Semi-quantitative image analysis of the DNA product generated by RT-PCR was performed to compare quantities of nasal RV detected on admission to ER and at week 6. Equal volumes of the RT-PCR reaction were electrophoresed through agarose gels and the intensity of each band was determined using GeneTools software. RSV was detected by RT-PCR as we have reported [17].

Measurements of peak expiratory flow

Peak expiratory flow (PEF) measurements were done as soon as patients were admitted to ER. Staff were trained in the use of PEF meters and they obtained three measurements prior to the first administration of β -agonist nebulization. Repeat measurements were done 10 min after treatment. Forty-four patients were able to provide three repeatable PEF measurements (within 10%); in six patients this could not be accomplished. Measurements were performed using a mini-Wright peak flow meter (Clement Clarke International, London, UK) and the highest of the three measurements was used for the study. For analysis all PEF measurements were expressed as % predicted (% pred).

Skin prick testing

Skin prick tests (SPTs) were performed on an abbreviated panel (house dust mite, SA grasses, cat dander) of common allergens (Bencard, Cape Town, South Africa) with appropriate controls. A positive result was defined as a reaction ≥ 3 mm to one or more allergens in the presence of positive histamine and negative saline controls. Previous studies in Cape Town have demonstrated that $>85\%$ of atopic individuals have a positive skin test response to at least one of the allergens listed above [18].

Statistical analysis

Our previous studies in adults detected RV RNA in 37% of adult patients with acute asthma [3] and because identification rates in children have been considerably higher [2, 4] we estimated that approximately 50–60% of children with acute asthma may have detectable RV RNA. Fifty patients provided $>80\%$ power to detect a 10% difference in predicted PEF (SD 15%) at the 5% level, comparing those patients with or without RV RNA.

The prevalence of RV was compared in ER, at 6 weeks, at 6 months and in controls using χ^2 analysis or Fisher's exact test as appropriate. For analysis of atopy and RV RNA as categorical variables, Fisher's exact test was employed. PEF measurements in ER and after 6 weeks and measurements in patients with and without persisting RV RNA were compared using unpaired *t*-tests. The impact of RV and atopy on PEF measurements was assessed employing multivariate regression analysis with PEF as dependent variable. Data are expressed as mean $\pm$ SD and $P < 0.05$ was considered significant.

Results

Virus detection

Virus detection on admission Overall RV RNA and RSV RNA were detected in 42 out of 50 asthmatic children (84%) admitted to an ER with acute severe asthma. RV RNA was present in nasal aspirates obtained from 41 (82%) subjects. Dual infections were detected in five children and only one child had RSV RNA without RV RNA also being present. Detection of RV is depicted in Fig. 1.

Virus detection after 6 weeks Patients were again assessed 6 weeks after ER admission. Nine subjects did not return (18%) of whom seven patients previously had RV RNA detectable in ER (Fig. 1). RT-PCR for RSV RNA was not repeated after 6 weeks because NAs were not available for this assay.

RV RNA was detected in 22 patients, in 18 RNA had been present in ER (44%, $P = 0.005$ vs. ER) and four subjects now had RV RNA after a negative RT PCR assay in ER (Fig. 1). In 16 subjects RV RNA, detected originally in ER, was not detectable after 6 weeks. In three patients, RV RNA was absent as it had been on admission to ER.

Image analysis of DNA (derived from the RT-PCR assay) that had been detected on gel electrophoresis revealed significantly higher image densities in ER compared with week 6 ($P = 0.0005$, Fig. 2) suggesting that, in the same patient, greater quantities of nasal RV RNA were likely to have been present in ER. In ER, there was no difference of RT-PCR assay DNA quantities in patients with and without persistence of RV RNA ($P = 0.3$).

Virus detection after 6 months To assess the prevalence of RV RNA in the study cohort a subgroup of patients ($n = 16$) were reviewed 6 months after admission to ER; all had a history of stable asthma over the preceding 3 months. RV RNA could be detected in four patients (25%, $P = 0.008$ vs. ER, NS vs. week 6), all of whom had RV RNA present initially in ER. Of the 12 patients without RV RNA at month 6, 6/12 had RV RNA detectable at week 6. There was thus a decrease over time of RV RNA detection in this group: 11/12

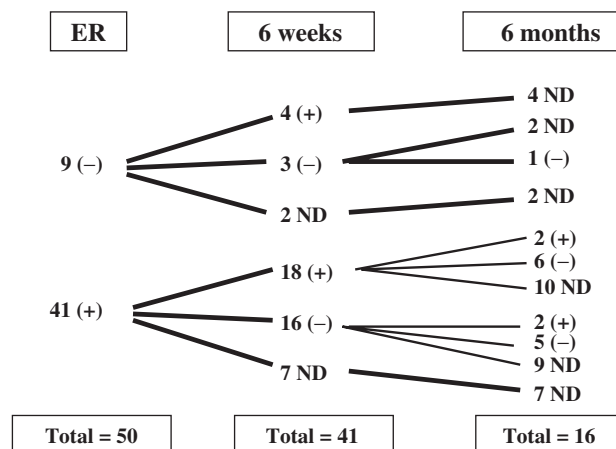


Fig. 1. Detection of rhinovirus RNA (+ or -) in the study cohort during emergency room (ER) admission, at week 6 and after 6 months is depicted. ND, not determined.

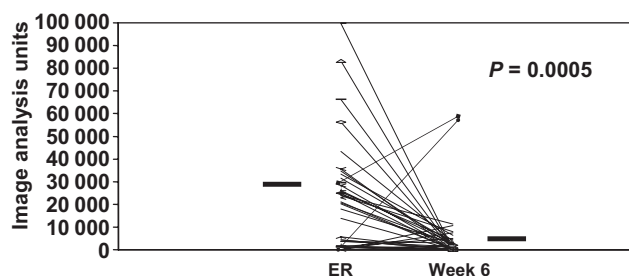


Fig. 2. Image analysis of DNA from the RT-PCR assay was performed on nasal aspirates (NA) samples obtained in emergency room (ER) and at week 6 and compared for each patient. There was significantly less DNA (reflecting rhinovirus (RV) RNA) present in NA samples from week 6, suggesting that residual RV RNA was present rather than fresh infection.

patients in ER, 6/12 at week 6 and 0/12 after 6 months (Fig. 1).

Virus detection in control asthmatics Nine stable asthmatic children were assessed at the same time (6 months) and from the same hospital clinics as the study cohort. RV RNA was detectable by RT-PCR assay in two of nine subjects (22%, $P = 0.009$ vs. ER, NS vs. week 6).

Clinical outcomes

Baseline demographic data are shown in Table 1. All patients lived in an urban area. A history of at least one previous ER admission for acute asthma was obtained from 48 patients and almost all subjects (49/50) gave a history suggestive of an antecedent URTI. There were no differences in the number of lower airway symptoms between patients with and without RV RNA or in the duration of the exacerbation prior to ER. This was also true for comparisons of subjects with or without RV RNA after 6 weeks (data not shown). The duration of OCS treatment was 5.5 ± 0.8 days in subjects with persistent RV RNA (accurate data were available in 10/18 patients) and 5.1 ± 1.2 days in those without persistence (data from 13/16 patients, $P = 0.47$). The number of patients hospitalized was reviewed and was also similar (10/18 in persistence vs. 8/16, $P = 0.80$).

All patients were treated with β -agonist nebulization and given a course of OCS as recommended in asthma management guidelines [13]. A clinical review conducted after 6 weeks suggested that asthma had been stabilized in the majority of patients although 11 patients (nine with RV RNA present) reported ongoing daily wheezing and/or night asthma. Clinical symptoms of a URTI [2] were reported by only one patient at the 6 week review.

PEF measurements

Comparison of patients with and without detectable RV RNA PEF measurements were compared to assess whether the presence of RV RNA may be associated with more severe exacerbations. In ER, PEF measurements were $63.4 \pm 16.4\%$ in all patients ($n = 44$), $63.4 \pm 16.5\%$ in those without RV RNA present ($n = 9$) and $62.1 \pm 16.7\%$ in patients with RV RNA ($P = \text{NS}$). Bronchodilator responses were $13 \pm 18.9\%$ in patients without RV RNA and $8.9 \pm 9.5\%$ in those with

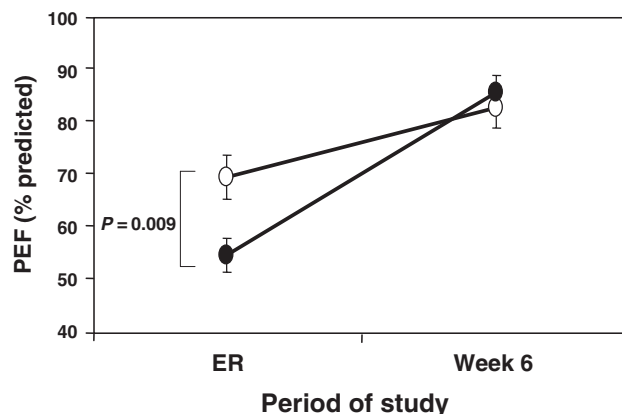


Fig. 3. Measurements of peak expiratory flow (PEF) in patients with ($n = 18$, closed circles) and without ($n = 16$, open circles) rhinovirus RNA persistence.

RV RNA present ($P = 0.47$). After 6 weeks, PEF was $85.3 \pm 20.8\%$ in the overall group, and a significant overall improvement ($P = 0.001$) but no differences in PEF measurements was found between subjects with RV RNA present or absent at week 6 ($P = 0.6$).

Comparison of patients with and without persistence of RV RNA To assess whether the persistence of RV RNA after 6 weeks influenced PEF measurements obtained in ER, subjects were compared as follows:

- RV RNA present in ER and absent at week 6 ($n = 16$, group A) or
- RV RNA present in ER and present at week 6 ($n = 18$, group B)

In group A, PEF measurements in ER were significantly higher ($69.9 \pm 16.7\%$) than in group B ($55.1 \pm 13.7\%$; $P = 0.009$). This difference disappeared at week 6 as shown in Fig. 3 ($86.1 \pm 12.5\%$ vs. $83.3 \pm 16.7\%$ respectively; $P = 0.6$). Bronchodilator responses in groups A and B (after 10 min in ER) were similar (group A: $7.7 \pm 7.4\%$, group B: $11.0 \pm 8.8\%$, $P = 0.49$). Improvements in PEF measurements at week 6 were significantly greater in patients in group B ($31.3 \pm 13.4\%$ vs. $14.7 \pm 18.9\%$ in group A; $P = 0.008$).

We also compared PEF measurements in patients with RSV to those in patients without RV or with RV. No differences were found (data not shown).

Association of positive skin prick responses to allergen with persistence of RV RNA

Association of atopy with RV RNA and persistence of RNA were examined using univariate analyses. Patient groups were defined by positive skin prick responses to allergen and detection of RV RNA. Overall, 37 of 50 children studied in ER had ≥ 1 positive SPT (atopy). From a total of 41 children with RV RNA detected while in ER, 28 were atopic, whereas the nine patients without detectable RV RNA were all atopic ($P = 0.09$). At week 6, 17 children (out of a total of 22 with RV RNA present) had both atopy and RV RNA present compared with 12 atopic children out of a total of 19 in whom RV RNA could not be detected ($P = 0.55$). The proportion of

atopic children with RV RNA present both in ER and at week 6 (13 of 18) was similar to those with RV RNA present in ER but not at week 6 (9 of 16, $P = 0.47$).

Multiple regression analysis did not reveal any effect of RV RNA or skin prick responses to allergen on PEF measurements in ER (atopy mean difference 1.5%, $P = 0.78$ and RV RNA 0.5%, $P = 0.9$). However, a combination of RV RNA persistence, atopy and PEF measurements into a multiple regression model indicated that the effect of RV RNA persistence on PEF in ER remained after controlling for the effects of atopy (mean difference 15.1%, $P = 0.009$). Positive skin prick responses did not predict PEF in ER after controlling for the effect of persistent RV RNA (mean difference 1.4%, $P = 0.8$).

Discussion

This study in children aged 4–12 years confirms the high prevalence of putative RV infection during asthma exacerbations. Comparable studies in children admitted to an ER [4] and in an outpatient cohort [2] have demonstrated that up to 70% of young asthmatics have detectable nasal RV RNA during episodes of airway obstruction. Our study conducted on a different continent has found an almost identical prevalence in children with moderate-to-severe airway obstruction and the results provide further evidence for the ubiquitous role of RV as a cause of acute asthma in children. Although this study did not attempt to identify other viruses, previous studies in the UK, USA and Australia have indicated that RV and RSV make up 80–95% of virus identifications and indicated that other viruses appear to play a very minor role [2, 4, 19].

An unanticipated result was that more than 40% of children still had RV RNA detectable after 6 weeks; previous studies have suggested a prevalence of 12–15% in asymptomatic asthmatic children [2]. Studies of comparable design to the present study have not been published although a recent report from Finland noted that 50% of infants (mean age 1.7 years) had RV RNA detectable 2 weeks after a wheezing episode [20]. Interestingly, one study found a relatively high prevalence of RV (36%) in children admitted to ER with non-respiratory illness, but studies examining the prevalence of RV in non-hospitalized patients in the same community were not reported [4]. The relatively high prevalence of RV RNA after 6 weeks found in the current study has two feasible explanations, residual infection with the same serotype or new infection with a different serotype. Our data cannot distinguish between these possibilities but the balance of evidence suggests that it is likely to reflect detection of the same RV RNA that had been identified initially in ER. Firstly, we used image analysis of the RT-PCR assay product to compare RV RNA in ER and at week 6 and found significantly lower quantities at week 6. It is unlikely that new, wild-type RV infections would be associated with significantly smaller quantities of virus RNA and hence our data appear to be most consistent with some reduction but not complete elimination of RV RNA after 6 weeks. Secondly, although RV RNA was detected after 6 weeks, it was not associated with fresh symptoms of a cold as

expected in the case of a new infection. PEF measurements were also not reduced in patients with RV RNA present compared with those without RV RNA. Thirdly, on restudy the prevalence after 6 months in a subgroup of the same cohort was 25%, similar to the rate in the control group of children with stable asthma. This is almost half the prevalence rate recorded after 6 weeks in the study. Finally, in the subjects who were 'free' of RV RNA after 6 months there was a stepwise decrease in RV RNA (11 of 12 patients with RV RNA in ER; six of 12 patients with RV RNA after 6 weeks; zero of 12 after 6 months) suggesting elimination of infection rather than new infections over time.

The period between infection by RV and elimination of the virus with a negative PCR assay is not known and this is the first report of RV RNA found in a considerable number of individuals for as long as 6 weeks after first detection. Gern et al. [12] have found that experimental RV infection in adults is cleared after 14 days in 50% of volunteers. Their study using an experimental infection model and a single serotype (RV16) in an adult population is evidently not comparable with wild-type infections detected in our study, but the results suggest that RV can be present in the nose for an extended period after acute infection. We have previously reported a low prevalence (<5%) of RV RNA 6 weeks after asthma exacerbation in adult asthmatics [3]. The difference may be related to younger age, greater cold severity or enhanced sensitivity of the RT-PCR assay in the current study. However, the RV RNA prevalence of approximately 20% after 6 months was comparable with other studies [2, 4]. To date it is not known whether factors such as atopy, asthma and age influence the duration of RV infections although it was noted that virus clearance was associated with increased IFN- γ /IL-5 ratios and hence a T-helper type 1 bias in experimental infections [12].

RV common colds are characterized by prominent nasal and upper airway symptoms and generally mild or absent systemic manifestations of infection. It has been assumed that the mild systemic nature of the host response reflects a relative absence of significant detrimental effects on other organs and pre-existing diseases. However, several studies have provided compelling evidence that RV induces asthma exacerbations and recent reports have suggested that RV infection may cause more severe lower airway disease than other viruses [10, 11]. The first aim of the studies presented here was to contrast the severity of RV and non-RV exacerbations employing assessments of PEF. The comparison was diluted by the small number of asthmatics without RV RNA. However, the observation that >40% of patients had persistence of RV RNA suggested that this factor may identify a different patient population and effects on PEF measurements. Comparison of the groups demonstrated that persistence of RV RNA was associated with significantly lower PEF measurements in ER. It reflected a clinically significant difference (mean 69.9% predicted without vs. 55.1% with RV RNA) and suggests one explanation for the range of asthma exacerbation severities observed in ER [5]. This finding was not matched by differences in the number or duration of lower airway symptoms, probably because clinical parameters may not be sensitive indicators in this context.

Although our study does not provide reasons for the greater severity in this group, a number of possible explanations appear feasible. The most likely explanation is that children with more severe infection (as reflected by greater reductions in PEF) took longer to eliminate infection. Factors such as atopy may co-determine with RV the severity of exacerbations and in this fashion influence the host's ability to resolve infection, but regression analysis did not suggest that this played a role. Alternatively, it has been speculated that virus infections may induce β_2 receptor dysfunction [21]. All children are likely to have used β_2 -agonists repeatedly prior to ER; indeed most patients are likely to have used reliever treatment while waiting in the triage area of the ER. The differences in PEF observed may thus reflect resistance to bronchodilator treatment associated with RV infection, possibly more so in severe infection. Bronchodilator responses in ER failed to support this idea and were not reduced in patients with persistence of RV RNA.

A number of studies have suggested that atopy may be linked to a propensity for the development of RV-induced wheezing and that allergens and viruses act together to exacerbate asthma [4, 22]. For example, Rakes et al. [4] found a high odds ratio linking evidence of atopy (such as a positive RAST, odds ratio 17) with RV-associated wheezing. We used skin prick testing to assess the presence of atopy in the study cohort. Skin prick testing is a reliable indicator of atopy in children and the panel of allergens used in the study has been shown to detect atopy in the majority of persons with the condition [18]. No association between a positive SPT and the presence of RV RNA was noted, suggesting that atopy measured in this fashion does not increase the odds of acute asthma associated with RV RNA. There was also no association between a positive SPT and the persistence of RV RNA after 6 weeks. We evaluated whether atopy and RV RNA were linked to PEF measurements and found that persistence of RV RNA (but not atopy) was a significant predictor of lower PEF measurements. It is possible that factors other than a positive SPT (for example, host levels of virus neutralizing antibody) are the prime determinants of severity [23, 24]. Our findings are similar to a recent Australian study which failed to find a link between acute severity, RV and positive SPTs [5]. The current study differed from the investigations of Rakes and co-workers in two key respects: we did not include a similar number of patients in ER and hence the study may have been underpowered. In addition, IgE may be a more appropriate measurement of atopy that reflects the increased odds of wheezing with RV more consistently.

The study is limited by a number of factors. The study lacked power to compare PEF measurements in ER between asthmatics with and without RV RNA because of the relatively small number of patients without RV RNA. It was also not possible to culture and serotype RV or sequence DNA after RT-PCR to resolve whether persistent or new RV infection was likely to be present. Quantitative RT-PCR assays may have provided more concise information about viral load in ER and after 6 weeks, but were not in use in our laboratory. We were also unable to investigate the prevalence of RSV after 6 weeks and thus to examine interactions with RV infection. Finally, although we demonstrate greater acute

asthma severity in a subgroup, the study fails to provide information about the mechanisms that explain the differences observed.

In summary, this study again confirms the high prevalence of RV RNA in acute asthma. It also demonstrates that the virus can be present for an extended period in a significant number of patients and documents an association with more severe acute exacerbations in this group. Further studies are needed to examine virus infection and its role in the severity of acute asthma exacerbation. Allergen and virus-specific immunological responses in the host asthmatic also require further investigation to clarify the complex interactions of viruses with their human hosts [24].

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