The Relationship of Rhinovirus-Associated Asthma Hospitalizations with Inhaled Corticosteroids and Smoking

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Background. Although rhinovirus (RV) respiratory infections trigger asthma exacerbations, the etiologic association between this virus and severe exacerbations, as well as the clinical characteristics of adults at risk for RV-associated asthma that necessitates hospitalization, have not been established.

Methods. During 1999–2003, we conducted a cohort study of 101 adults prospectively enrolled at hospital admission for an asthma exacerbation. Patient characteristics and frequencies of RV in nasal specimens were analyzed, by reverse-transcription polymerase chain reaction (RT-PCR), at asthma-related hospital admission and at a 3-month convalescent follow-up visit.

Results. RV was detected by RT-PCR in 21% of hospitalized patients over a 4-year period and in 1.3% of patients who returned for a 3-month follow-up visit. RV detection was strongly associated with hospitalization for asthma (adjusted odds ratio [OR], 15.1 [95% confidence interval {CI}, 1.88–121.4]). After adjustment for baseline asthma severity, RV-positive patients were more likely than RV-negative patients to be current smokers and nonusers of inhaled corticosteroids (ICSs) (adjusted OR, 11.18 [95% CI, 2.37–52.81]; P = .002).

Conclusions. RV respiratory infection is an etiologic agent in severe asthma exacerbations necessitating hospitalization in adults. Compared with hospitalized patients with asthma who were RV negative, RV-positive patients were significantly more likely to be smokers and nonusers of ICSs.

Infections with common respiratory viruses frequently trigger exacerbations of asthma [1–13]. Early studies investigating this relationship employed viral culture techniques and found that the frequency of virus-associated asthma exacerbations was ~40% [1, 2]. Newer molecular techniques have demonstrated that viruses are associated with 80%–85% of asthma exacerbations in children and up to 60% of those in adults [3, 4].

Rhinovirus (RV), a picornavirus, is the respiratory virus that has been most frequently associated with asthma exacerbations [3–6, 8, 10, 11]. However, studies implicating this virus have primarily assessed viral detection at a single time point and have been performed in nonhospitalized patients with asthma. Fewer investigations have examined the relationship between RV and asthma exacerbations that are severe enough to require hospitalization. Johnston et al. have demonstrated that correlations exist between seasonal patterns of upper respiratory tract infections and hospital admissions for asthma in both children and adults [6]. However, no prospectively designed study that directly examines the role of RV in severe asthma exacerbations...
has been performed to confirm these results. Additionally, no studies to date have examined the clinical characteristics of adults admitted to the hospital with an RV-associated asthma exacerbation. The purpose of the present study was to evaluate the association between RV infection and asthma exacerbations precipitating hospital admission, by means of reverse-transcription (RT) polymerase chain reaction (PCR) detection at 2 different time points—hospital admission and a 3-month convalescent follow-up visit—and to characterize clinical features of at-risk patients.

**METHODS**

**Study setting and participants.** Five days per week during a full 4-year period, patients 18 years of age and older who were hospitalized at Vanderbilt University Medical Center (VUMC; Nashville, TN) with a diagnosis of acute asthma were approached for study inclusion. Review of hospital charts and prior medical records and consultation with the currently treating hospital physician(s) were used to confirm the diagnosis of asthma and to exclude other diagnoses, such as chronic pulmonary disease. Patients were excluded if they had concurrent congestive heart failure or another chronic pulmonary disease that could account for the acute illness or if they had previously been enrolled. Criteria for the diagnosis of acute asthma included confirmation of asthma diagnosis by review of prior records and current admission consistent with asthma disease exacerbation, including currently treating physician clinical diagnosis of asthma, presence of dyspnea, wheezing, and respiratory distress not attributable to another cardiopulmonary process.

Of 140 eligible adults approached on surveillance days, 101 (72%) were enrolled. Of the 39 not enrolled, 20 declined participation, and for 19 consent or surrogate consent could not be obtained. Seventy-six (75%) of the enrolled patients returned for the 3-month follow-up visit. The study was approved by the VUMC Institutional Review Board, and patients gave informed consent. Human experimentation guidelines of the US Department of Health and Human Services and those of Vanderbilt University School of Medicine were followed in the conduct of this research.

**Data collection.** A study nurse collected information on clinical symptoms, medical history, pediatric household contacts, and detailed smoking and environmental tobacco exposure; obtained spirometry, vital signs, nasal lavage specimens, and serum for IgE determination; and completed a physical assessment. Patients were followed daily during hospitalization. Chronic asthma disease severity was measured using the Johns Hopkins asthma severity scale, on which lower values indicate milder disease [14]. Patients were seen as outpatients in the General Clinical Research Center 3 months after their acute asthma exacerbation, where they underwent a similar comprehensive evaluation and skin-prick testing for 9 common aeroallergens.

**RV infection determination.** Cells and mucus were concentrated into the bottom of an eppendorf tube from nasal lavage samples by centrifugation for 15 min at 4°C. The supernatant was removed, except for the final 100 μL. RNA was extracted from the cells and mucus pellet with TRIzol Reagent (Gibco-BRL; Sigma), as described elsewhere [15]. The RNA was reverse transcribed by use of Superscript II reverse transcriptase (Invitrogen) in the presence of random primers (Promega), as described elsewhere [15]. For the first PCR, a “touchdown” reaction cycle was used [16, 17]. The primers (upstream, 5′-CGGACACCCAAAAGTAG-3′; downstream, 5′-GCACCTCTGTGTCCC-3′) amplify a 380-bp region in the 5′-untranslated region of picornaviruses, including RVs [15]. The second PCR was performed as described elsewhere [15]. The primers (the same downstream primer as in the first PCR; and RV nested, 5′-GCGAGCCACGC-AGGCT-3′) amplify a 202-bp region specific for the RV group. The sizes of the PCR products were verified by agarose electrophoresis; the results were not quantitative and were instead only an indication of the presence or absence of RV. Controls in each PCR run included samples containing reagents with no cDNA and a sample containing cDNA prepared from human RV type 16 (HRV16) RNA [15–17].

The detection sensitivity of our nested PCR assay was 100 RV particles/sample (1 infectious unit of RV contains 200–400 virus particles). The sensitivity was assessed with cDNA samples prepared from a known amount of HRV16 virus particles by standard extraction and a cDNA synthesis procedure. Briefly, sucrose density gradient–purified HRV16 virus particles were generated and then quantified optically, under the assumption of 0.133 μg (9.4 × 10^13 virions) per OD260 unit, as described elsewhere [18]. These virus particles were serially diluted in PBS. RNA was extracted, and cDNA was synthesized from each dilution for nested PCR assay, as described above. The results showed that samples containing >100 virus particles per extraction yielded visible 200-bp PCR products in an agarose gel.

**Tobacco exposure.** Second-hand smoke exposure and current smoking were defined and quantified by personal report and/or elevated urinary cotinine level, as determined by gas chromatography (National Medical Services, Willow Grove, PA). Patients with urine cotinine levels >200 ng/mL were categorized as current smokers, and those with levels of 1–199 ng/mL were categorized as exposed to environmental tobacco smoke, regardless of self-reported smoking status [19–21].

**Outcomes.** The major outcomes of interest were (1) the number of patients with human RV infection at each of 2 time points and (2) the characteristics of patients with RV-associated asthma exacerbation.

**Statistical analysis.** Point prevalences of RV infection during hospitalization for asthma and at a 3-month convalescent visit were calculated. Baseline characteristics were compared using χ² tests (or Fisher’s exact tests) for categorical variables and
Table 1. Demographic and asthma characteristics, including clinical presentation, disease severity, and spirometry, among 101 adult patients hospitalized for acute asthma, by rhinovirus (RV) infection status.

| Characteristic                                      | RV positive (n = 21) | RV negative (n = 80) | P     |
|----------------------------------------------------|----------------------|----------------------|-------|
| Age, mean ± SD, years                              | 36.3 ± 9.0           | 43.3 ± 11.2          | .007  |
| Sex                                                |                      |                      | .07   |
| Female                                             | 20 (95.2)            | 60 (75)              |       |
| Male                                               | 1 (4.8)              | 20 (25)              |       |
| Racea                                              |                      |                      | .49   |
| Black                                              | 8 (38.1)             | 38 (47.5)            |       |
| White                                              | 12 (57.1)            | 40 (50)              |       |
| Educationb                                         |                      |                      | .83   |
| Less than high school                              | 1 (4.8)              | 4 (5)                |       |
| High school/GED                                    | 10 (47.6)            | 34 (42.5)            |       |
| More than high school                              | 9 (42.9)             | 42 (52.5)            |       |
| Smoking status                                     |                      |                      | .002  |
| Current smoker                                     | 13 (62)              | 20 (25)              |       |
| Nonsmokerc                                         | 8 (38)               | 60 (75)              |       |
| Use of ICS                                         | 12 (57)              | 68 (85)              | .02   |
| Actuations per day of β-agonist, mean ± SD         | 2.6 ± 2.0            | 2.2 ± 1.2            | .54   |
| Chronic asthma severity score, score ± SD²         | 42.6 ± 21.9          | 71.2 ± 46.3          | .005  |
| History of hospitalization for asthma exacerbation | 9 (43)               | 60 (75)              | .005  |
| Three-month follow-up convalescent FEV₁, mean ± SD, % of predicted | 85.0 ± 10.5          | 67.0 ± 24.0²         | .02   |
| Upper respiratory tract symptoms                   |                      |                      |       |
| Fever                                              | 15 (71)              | 41 (51)              | .10   |
| Rhinorrhea                                         | 17 (81)              | 52 (65)              | .18   |
| Sore throat                                        | 12 (57)              | 48 (60)              | .81   |
| Congestion                                         | 18 (86)              | 56 (70)              | .07   |
| Admission FEV₁, mean ± SD, % of predicted          | 52.4 ± 16.5          | 54.6 ± 23.4          | .90   |
| FEV₁ change between hospitalization and 3-month follow-up visit | 26.5 ± 18.6          | 10.2 ± 18.9          | .02   |
| Total serum IgE level, median (range), IU/mL       | 107.5 (18.5–502)     | 60.3 (2–942)         | .10   |
| Skin prick test at 3-month follow-up visit         |                      |                      | .09   |
| Positive                                           | 10 (47.6)            | 41 (51.3)            |       |
| Negative                                           | 0 (0)                | 12 (15)              |       |
| Missing                                            | 11 (52.4)            | 27 (33.7)            |       |

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid.

a One patient in RV-positive group and 2 patients in the RV-negative group were both nonwhite and nonblack.
b Data were missing for 1 patient in the RV-positive group.
c Nonsmokers include former smokers, those exposed to environmental tobacco smoke, and people who have never smoked.
d Johns Hopkins Asthma Severity Score. Higher scores indicate more-severe disease.

the Wilcoxon rank-sum test to compare continuous variables. Adjusted odds ratios (ORs) were obtained by the use of a generalized estimating equation model to determine independent associations between RV infection at hospitalization and the 3-month follow-up visit, after controlling for baseline characteristics. To achieve adequate regression coefficient power, the first principal component was used to account for the variation of age, current smoking status, and sex as covariates. ORs and 95% confidence intervals (CIs) for smoking and use of inhaled corticosteroids (ICSs) were obtained from logistic regression models.
adjusted for asthma severity. Collinearity was checked and was not found among smoking and use of ICS. Models were additionally adjusted for the first principal component of age, sex, and asthma severity score, but the results did not differ significantly from the model adjusted for asthma severity (data not shown). Tests for interaction between smoking and use of ICSs were performed by calculating the log-likelihood difference between models with and without cross-product terms.

For all tests performed, a 2-sided significance level of 5% was used for inference. SAS (version 8.2; SAS Institute) was used. All reported $P$ values are unadjusted for multiple tests.

**RESULTS**

**Demographics of the entire cohort.** Among the 101 individual adult patients hospitalized with acute asthma exacerbation, 80% were female, the mean age was 41.9 years, 51.5% were white, 45.5% were black, and 51% had attained greater than a high school education. Most patients (62%) received medical care from a primary care physician alone, 51.5% of patients were users of ICSs, and 32.7% were current smokers.

**Detection of RV.** A nasal wash sample was found to be positive for RV by RT-PCR at hospital admission in 21 patients (21%). Seventy-six (75%) of the 101 patients returned for the 3-month convalescent follow-up visit. Of those returning for the 3-month visit, 1 patient (1.3%) was found to be RV positive by RT-PCR. That patient had nasal congestion, rhinorrhea, chest tightness, wheezing, and a forced expiratory volume in 1 s (FEV$_1$) that was 31% of predicted at the time of follow-up. Twelve (57%) of 21 patients positive for RV at admission returned for their 3-month follow-up visit, none of whom tested positive for RV. RV detection was strongly associated with hospitalization for asthma (adjusted OR, 15.1 [95% CI, 1.88–121.4]).

Patients who returned for the 3-month follow-up visit did not differ from nonreturning patients with respect to age, sex, race, daily $\beta$-agonist use, or length of hospital stay. However, those who did not return were more likely to smoke, less likely to have a college education, and less likely to use ICSs.

**Demographics and characteristics of RV-positive patients.** Compared with RV-negative patients, RV-positive patients hospitalized for asthma were more likely to be younger (mean ± SD age, 36.3 ± 9.0 vs. 43.3 ± 11.2 years; OR, 6.0 [95% CI, 1.64–22.98]; $P = .007$), be female (95% vs. 75% female; OR, 6.7 [95% CI, 0.84–52.9]; $P = .07$), be current smokers (OR, 4.88 [95% CI, 1.77–13.46]; $P = .002$), be nonusers of ICSs (OR, 4.26 [95% CI, 1.47–12.5]; $P = .02$), and have lower chronic asthma severity scores (mean ± SD, 42.6 ± 21.9 vs. 71.2 ± 46.3; $P = .005$) and were less likely to have been previously hospitalized for asthma exacerbation (43% vs. 75%; $P = .005$) (table 1). Between patients with and patients without RV infection, there were no differences noted in race, highest degree of education attained, or mean number of daily $\beta$-agonist actuations. Symptoms of upper respiratory tract infection and FEV$_1$, at the time of admission did not differ between RV-positive and RV-negative patients, but, at the 3-month follow-up visit, FEV$_1$ was significantly higher among patients who had tested positive for RV infection (85% ± 10.5% vs. 67% ± 24.0% predicted, respectively; $P = .02$); thus, between the time of hospitalization and the 3-month follow-up visit, the change in FEV$_1$ was greater in RV-positive patients than in RV-negative patients (26.5% vs. 10.2%; $P = .02$).

RV-positive patients had relatively high total serum IgE levels at admission, but these levels were not significantly different than those in RV-negative patients. At the follow-up visit, all available RV-positive patients had a positive reaction to at least 1 inhalant allergen by skin-prick testing. Thirty-eight patients were missing skin test data, and no statistically significant differences between groups were noted.

There were no significant differences between the groups with regard to the type of physician treating asthma, length of asthma-related hospital stay, change in FEV$_1$ during the first 24 h of hospitalization for asthma or entire length of stay, discharge FEV$_1$, or number of days in the intensive care unit (ICU) for those with ICU stays (data not shown). There were no significant differences in whether patients with or without RV infection lived with children or elderly individuals who attended day care, lived with children who attended school, had exposure to children...
Figure 1. Seasonality of rhinovirus (RV) isolation from December 1999 to December 2003, by month. The enrollment period was from 6 December 1999 through 11 December 2003.

in their work environment, or a combined variable incorporating all of these exposures (data not shown).

In the univariate analyses, RV-positive patients were more likely to smoke and to be nonusers of ICSs. The combination of these 2 factors was strongly associated with RV positivity (OR, 11.18 [95% CI, 2.37–52.81]; \( P = .002 \)) (table 2).

Seasonality of admissions. Seasonality of RV admissions was determined for the 4-year study period and is depicted by month for the combined 4 years; circulation of RV throughout the year can be seen, with peaks in the spring (April and May) and fall (September–December). RV accounted for the greatest proportion of hospitalizations for asthma in September and the greatest absolute number of admissions in May (figure 1).

DISCUSSION

We have shown an etiologic association between RV infection and adult asthma exacerbations necessitating hospital care and have identified clinical characteristics of patients with RV infection. In this cohort, female sex, younger age, current smoking, and ICS nonuse characterized patients hospitalized for RV-associated asthma.

Previous clinical studies have suggested that RV plays a role in asthma exacerbations, but these investigations assessed RV infection at a single time point, and their interpretations are complicated by the high prevalence of RV positivity in asymptomatic control subjects [3, 4, 8, 22]. Ours is the first study, to our knowledge, to assess the etiologic role of RV in severe asthma exacerbations necessitating hospital admission by assessing viral detection, through use of RT-PCR at hospitalization and at a 3-month follow-up visit, in the same study subjects. RV infection was associated with 21% of adult asthma admissions over the 4-year study period and was strongly associated with hospitalization for asthma (OR, 15.1 [95% CI, 1.88–121.4]).

Other investigations have shown an association between RV infection and asthma exacerbations in several populations. El-Sahly et al. found that 93% of patients 5–35 years of age who were admitted with an acute respiratory illness associated with a “common cold” virus—either RV or coronavirus—had asthma [23]. Two other investigations of adults attending an emergency room with asthma exacerbations detected viruses in the sputum of 70%–76% of patients, with one of the studies reporting that RV accounted for 83% of the viruses identified [24, 25]. Two
prior investigations of hospitalization for RV-associated severe asthma in adults found RV infection prevalences of 11% and 35%, respectively [7, 9]. The first study used a viral culture technique, which is less sensitive than PCR, and the second study was a 12-month study in which no subjects were regular smokers and was not designed to perform a statistical comparison of RV detection at separate time points. Two additional investigations of the association between viral upper respiratory tract infections and severe asthma exacerbations in adults reported picornavirus infection prevalences of 26% and 28% but did not provide specific data regarding RV [13, 26]. The peak prevalence of RV-associated hospitalization in this adult population was noted in September, similar to the September peak described in children [11, 27].

The clinical characteristics of patients hospitalized for RV-associated asthma have not been previously described. In the present study, RV-positive patients had relatively mild baseline asthma, as evidenced by their normal 3-month follow-up/convalescent FEV1, greater reversibility of lung function, significantly lower frequency of ICS use at baseline, and lower chronic asthma severity scores, compared with those in RV-negative patients [14]. Patients with RV infection also were less likely to have a history of hospitalization for an asthma exacerbation.

Patients with RV infection were significantly less likely to be ICS users. The potential beneficial effects of ICSs in viral exacerbations of asthma was recently reported in a study of the September epidemic of asthma in children [11]. Johnston et al. found that children who required emergency room treatment for asthma were less likely to have had ICSs prescribed than were children with asthma in the same communities who did not require emergency care [11]. ICSs are potent anti-inflammatory agents that have been demonstrated to ameliorate abnormalities of the bronchial epithelium in patients with asthma and of the nasal mucosa in those with allergic rhinitis [28]. Injury to the airway epithelium and a differential phenotype of asthma epithelium are likely to be important risk factors for RV-associated asthma, as demonstrated by differential infection by RV of normal and disrupted or asthmatic airway epithelium [29, 30]. Additionally, studies have shown that ICS use dramatically reduces rates of hospitalization for asthma in children, of which as many as 85% are known to be associated with viral infections [31, 32].

Patients with RV infection were also significantly more likely to be smokers. The increased risk of RV infection in smokers with asthma has biological plausibility, because the role of tobacco smoke as a risk factor for a variety of respiratory infections has been investigated, and cigarette smoking has previously been identified as a risk factor for community-acquired pneumonia [33–36]. In addition to being a risk factor for the acquisition of certain respiratory infections, cigarette smoking has also been identified as a risk factor for lower respiratory tract complications of RV infection in a cohort of older adults, 63–90 years of age [37]. The experimental evidence to date also suggests that the airway epithelium is a site of damage from smoking, and investigations into the effect of cigarette smoke on the airway epithelium have revealed that chronic smokers have increased air-space epithelial permeability, which could contribute to host susceptibility to infection [38]. In experimental models, RV also more easily infects undifferentiated cells [29]. Additionally, because neutrophils are the predominant inflammatory cell associated with both respiratory viruses and tobacco use, smoking may well be a host factor predisposing to RV-associated asthma exacerbations by priming of lower airway neutrophilic immunopathologic effects [39–41]. The findings that as many as 35% of patients with asthma who present to the emergency department with acute asthma smoke tobacco and that 60% of adult asthma exacerbations are associated with viral infections serve to illustrate the importance of understanding the relationship between tobacco smoke and respiratory viral infections in patients with asthma exacerbations [4, 42].

Both smoking and nonuse of ICSs were characteristics of adults hospitalized with RV-associated asthma exacerbations (adjusted OR, 11.18 [95% CI, 2.37–52.81] vs. RV-negative patients; P = .002), and ICS use modifies the risk of RV infection even when adjusted for asthma severity. This interaction between smoking and ICS use deserves further exploration, since ICSs may affect RV-induced illness by restitution of the airway epithelium, as well as modification of host response to virus, and this may be different for smokers and nonsmokers. Tobacco cessation may have an even more important role in modifying the outcome of RV infection among persons with asthma.

There are several potential limitations of this study to consider. First, the study is relatively small and was conducted at a single university medical center, and our findings may not be generalizable to other populations. Other studies have detected higher RV infection prevalences during adult asthma exacerbations; however, one other study that used nasal specimens yielded an RV prevalence similar to that in our study, suggesting that the relatively higher rates of viral detection in those investigations may be explained by potential differences in detection rates between nasal and sputum specimens [43]. However, because we used the same methodology to compare patients at both time points, the conclusions drawn should be valid. In addition, we were only able to obtain convalescent specimens from 75% of study patients, including 12 who were RV positive at hospital admission. Although there were no significant differences in general demographic characteristics between study patients who did and those who did not follow-up that would suggest that RV infection rates may be differential, because study patients who did not return for follow-up were more likely to smoke and because smoking appeared to be a characteristic of those with RV infection, convalescent-phase RV infection prev-
The prevalence of RV infection at the convalescent follow-up visit (1.3%) was lower than that previously reported for asymptomatic populations, and the single individual in this study in whom it was detected at follow-up had symptoms of a respiratory infection and significantly exacerbated asthma. Previous reports of RV detection in asymptomatic individuals have varied from 10% to 12% in subjects with asthma and from 4% to 35% in subjects without asthma, with studies varying in the length of time that subjects had no upper respiratory tract symptoms preceding sample collection [3, 4, 8, 22]. Three of these studies, however, were performed in children with asthma, and a recent study by Jartti et al. revealed that, after the onset of symptomatic respiratory infection, RV RNA may take 5–6 weeks to disappear from the nasopharynx of children admitted with acute respiratory wheezing [44]. Lastly, the etiology of exacerbations in the RV-negative group was not determined, but a large proportion were almost certainly caused by other respiratory viruses, as evidenced by a high prevalence of upper respiratory tract symptoms.

In summary, these data confirm that RV respiratory infection is an etiologic factor for severe asthma exacerbations necessitating hospitalization, and patients with RV infection appear to have unique characteristics, including current smoking and nonuse of ICs. The role of smoking and ICs in host susceptibility to viral infection in asthma deserves further exploration, since this has significant implications for indications for ICs use and in decreasing severe asthma exacerbations and disease morbidity. The finding that 21% of hospitalizations for severe asthma are associated with RV positivity can be considered significant, in view of the lack of licensed antivirals for RV and the unlikelihood, because of multiple serotypes, of a vaccine being developed. The importance of RV infection in this setting illustrates the need for further research.

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