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Asthma is the most common chronic respiratory disorder in childhood, affecting about 10% of local children. Asthma exacerbation resulting in hospitalization accounts for a major fraction of the total cost of asthma care in society. Prospective epidemiologic studies show that up to 80% of childhood asthma exacerbations are associated with viral upper respiratory tract infections (RTIs). Among respiratory viruses, human rhinovirus (HRV) infection preceded as many as 50% of asthma exacerbations in children. Influenza viruses also accounted for substantial morbidity (e.g., up to 200 annual outpatient visits per 1,000 children) in children with asthma and other chronic medical conditions. Respiratory syncytial virus (RSV) is another major pathogen causing asthma exacerbation.

Background: Up to 80% of asthma exacerbations in white children are associated with viral upper respiratory infections. The relative importance of different respiratory pathogens and relevant microbiological data in Asian children are unclear. This study elucidated the epidemiology of respiratory infections in Hong Kong children with asthma exacerbation.

Methods: A total of 209 children aged 3-18 years with asthma exacerbations and 77 controls with stable asthma were recruited. The severity of asthma exacerbations was assessed according to Global Initiative for Asthma guideline, and subjects aged 6 years or older performed exhaled nitric oxide and spirometric measurements. Nested multiplex polymerase chain reaction was used to detect 20 different respiratory pathogens.

Results: Respiratory pathogens were detected in 105 (51.0%) subjects. The presence of any respiratory pathogen was associated with asthma exacerbation (odds ratio [OR], 2.77; 95% CI, 1.51-5.11; P < .001). Specifically, human rhinovirus (HRV) infection was more common among children with asthma exacerbation (OR, 2.38; 95% CI, 1.09-5.32; P = .018). All other pathogens or coinfections were not associated with asthmatic attacks. None of these respiratory infections was associated with the severity of asthma exacerbation (P > .15 for all). During peak HRV season in the winter of 2007 to 2008, this virus was detected in 46.4% of children with asthma exacerbations.

Conclusions: Respiratory viral infections are commonly found in children with asthma exacerbation, with HRV being the most important pathogen in our patients. Respiratory viral infection is a triggering factor for asthma exacerbation but does not correlate with its severity.
Respiratory viruses, such as human coronavirus (HCoV)-229E, HCoV-OC43, and human metapneumovirus (HMPV), are common causes of upper RTI, but their relation to asthma exacerbation remains uncertain. Allander et al developed a system for large-scale molecular virus screening of clinical samples based on host DNA depletion, random polymerase chain reaction (PCR) amplification, large-scale sequencing, and bioinformatics. A more recently described parvovirus called human bocavirus (HBoV) was identified in children with lower RTIs, but its relation to asthma exacerbation is uncertain at present.

Asthma exacerbation may also be caused by atypical bacteria. Chlamydia pneumoniae, an obligate intracellular respiratory pathogen, was linked to its relation to asthma exacerbation is uncertain at present.

In the study. These young patients also showed good response to shortness of breath, and wheezing during the 12 months before to methacholine or showed reversible airflow limitation, whereas positive for improved in half of adult patients with asthma sero-

Asthma diagnosis was made according to British Thoracic Society criteria. Briefly, older patients were either hyperresponsive to methacholine or showed reversible airflow limitation, whereas young children with asthma had three or more episodes of cough, shortness of breath, and wheezing during the 12 months before the study. These young patients also showed good response to bronchodilator. This study selected only children older than 3 years with a definitive diagnosis of asthma because it may be difficult to differentiate acute bronchiolitis from asthma in the younger children. Patients who received antimicrobial agents (eg, neurnadinase inhibitors, ribavirin, and macrolides) within 2 weeks before study were also excluded. The severity of asthma exacerbation was classified according to Global Initiative for Asthma guidelines.

Infection was defined as the detection of respiratory pathogens by our nested multiplex PCR assays. Our primary outcome was the difference in detection rate for any respiratory pathogen between children with asthma with acute exacerbation and controls (ie, stable asthma). Secondary outcomes consisted of differences in the clinical severity of asthma exacerbation, lung function parameters, and fractional exhaled nitric oxide concentration (FeNO) in relation to patients with different respiratory pathogens.

### Materials and Methods

#### Study Population

This study recruited children with asthma aged 3-18 years with disease exacerbation who received treatments either in pediatric wards or outpatient clinics of a university teaching hospital between January 2007 and February 2008. Inpatients hospitalized for asthma exacerbation were assessed within 48 h of hospitalization. The mean FeNO measurement using a chemiluminescence analyzer (Sievers; Boulder, CO) according to international guidelines. The mean FeNO of three NO plateau values was recorded. FeNO was measured within 48 h of hospitalization for children with acute asthma and at the clinic visit for stable patients. The former group was allowed to commence systemic corticosteroids as clinically indicated prior to FeNO because it would be unethical to withhold such treatment until this study. Following FeNO, they performed spirometry (Compact II; Vitalograph; Buckingham, UK) to measure PEV, FVC, and FEV/FVC.

#### Microbiological Investigations

In accordance with local Infection Control policy, nasopharyngeal aspirates (NPAs) were collected in negative-pressure isolation rooms. Deep nasal swabs were obtained as an alternative in situation where an isolation facility was unavailable. These specimens were put immediately in viral transport medium and kept at 4°C during transportation. Both viral RNA and DNA were extracted on the same day of collection by PureLink Viral RNA/DNA Mini Kit (Invitrogen; Carlsbad, CA). RNA extracted was converted to cDNA by reverse transcriptase (Superscript III Reverse Transcriptase; Invitrogen). All DNA and cDNA were used immediately for nested multiplex PCR for 20 respiratory pathogens as described previously.

#### Exhaled Nitric Oxide and Spirometric Measurements

Subjects 6 years of age and older underwent online FeNO measurement using a chemiluminescence analyzer (Sievers; Boulder, CO) according to international guidelines. The mean FeNO of three NO plateau values was recorded. FeNO was measured within 48 h of hospitalization for children with acute asthma and at the clinic visit for stable patients. The former group was allowed to commence systemic corticosteroids as clinically indicated prior to FeNO because it would be unethical to withhold such treatment until this study. Following FeNO, they performed spirometry (Compact II; Vitalograph; Buckingham, UK) to measure PEV, FVC, and FEV/FVC.
included for each group simultaneously. In order to prevent PCR contamination, reagent preparation, sample processing, and nested PCR assays were performed in separate rooms away from where amplified products were analyzed. Aerosol-resistant pipette tips were used throughout the experiments.

**Statistical Analysis**

As RTIs are age-dependent, we tried to match one control per patient with respect to their age and sex. However, we failed to recruit this target number of controls because many children with stable asthma had RTI symptoms during winter. The detection rates for respiratory pathogens between cases and controls were analyzed by χ² or Fisher exact test. The severity of asthma exacerbation, FeNO, and spirometric parameters were analyzed between subgroups with different pathogens by χ² or Student t test. Multivariate logistic regression was used to identify respiratory pathogens associated with asthma exacerbation, adjusted for age, inhaled corticosteroid (ICS) treatment, and domestic tobacco smoke exposure as covariates. All analyses were performed two-tailed using SPSS v.14 (SPSS Inc.; Chicago, IL), with the level of significance set at .05.

**RESULTS**

Two hundred nine children with asthma exacerbation, including 203 patients hospitalized in our pediatric wards and six who attended our outpatient clinics, and 77 controls with stable asthma were recruited. Table 1 shows the characteristics of these patients. Children with asthma exacerbation were younger than the controls, mainly because of our inability to recruit one age-matched control for each child with asthma exacerbation. Similar proportions of patients in the two groups received regular ICS treatment.

Sufficient respiratory samples were collected from 206 (98.6%) cases and all controls; these consisted of 236 NPA samples and 47 nasal swabs. Respiratory pathogens were detected in 105 (51.0%) subjects. Table 2 summarizes the distributions of respiratory pathogens in two groups of patients. The presence of any virus with or without atypical bacteria was associated with asthma exacerbation (P < .001 for both). Specifically, HRV infection was more common among children with asthma exacerbation (odds ratio [OR], 2.38; 95% CI, 1.09-5.32; P = .018). On logistic regression, asthma exacerbation was associated with the detection of HRV (OR, 2.36; 95% CI, 1.11-5.00; P = .025), any respiratory virus (OR, 2.19; 95% CI, 1.17-4.08; P = .014), or any respiratory pathogen (OR, 2.15; 95% CI, 1.16-4.00; P = .015). None of the respiratory pathogens or their coinfection was associated with the severity of asthma exacerbation (P > .15 for all). Age, gender, and ICS treatment did not affect the detection of respiratory pathogens in patients with asthma exacerbation (P > .1 for all).

Table 3 summarizes the clinical features of patients with asthma exacerbation in relation to HRV infections.

### Table 1—The Clinical and Objective Features of the Two Patient Groups

| Feature                              | Asthma Exacerbation (n = 209) | Stable Asthma (n = 77) |
|--------------------------------------|------------------------------|-----------------------|
| Age, y                               | 7.6 (4.1)                    | 11.1 (4.5)            |
| Men                                  | 68.4%                        | 75.3%                 |
| Duration of hospitalization, d       | 3.6 (1.9)                    | NA                    |
| Domestic tobacco smoke exposure      | 22.3%                        | 13.0%                 |
| Received regular ICS treatment       | 19.7%                        | 24.7%                 |
| Critical status                      |                              |                       |
| Fever                                | 33.0%                        | NA                    |
| Shortness of breath on talking/rest  | 8.7%                         | NA                    |
| Only able to talk in phrases or words| 11.2%                        | NA                    |
| Altered consciousness (agitation or drowsiness) | 0 | NA |
| Duration of fever, d                | 0.49 (0.86)                  | NA                    |
| Received supplemental oxygen         | 23.0%                        | NA                    |
| GINA-defined severity of exacerbations |                |                       |
| Mild                                 | 5 (2.4%)                     | NA                    |
| Moderate                             | 101 (48.3%)                  | NA                    |
| Severe                               | 103 (49.3%)                  | NA                    |
| Imminent respiratory arrest          | 0                            | NA                    |
| Vital signs                          |                              |                       |
| Minimum SaO₂, %                      | 94.1 (2.4)                   | NA                    |
| Maximum pulse rate, per min          | 135 (22)                     | NA                    |
| Maximum respiratory rate, per min    | 34 (9)                       | NA                    |
| Systolic blood pressure, mm Hg       | 110 (15)                     | NA                    |
| Diastolic blood pressure, mm Hg      | 69 (10)                      | NA                    |
| Laboratory results                   |                              |                       |
| FeNO, ppb                            | 57.2 (43.0)                  | 77.2 (59.6)           |
| FEV₁, % predicted                    | 73.2 (21.7)                  | 95.8 (15.2)           |
| FVC, % predicted                     | 81.6 (32.5)                  | 94.2 (18.8)           |
| FEV₁/FVC                             | 0.81 (0.25)                  | 0.86 (0.10)           |
| PEF, L/min                           | 194 (79)                     | 344 (132)             |
| Outcomes                             |                              |                       |
| Received systemic corticosteroid     | 75.4%                        | NA                    |
| ICU care                             | 3.5%                         | NA                    |
| Death                                | 0                            | NA                    |

Results expressed in mean (SD) unless stated otherwise. FeNO = fractional exhaled nitric oxide concentration; GINA = Global Initiative for Asthma; ICS = inhaled corticosteroid; NA = not available or applicable; PEF = peak expiratory flow; SaO₂ = arterial oxygen saturation.  
*Among children aged $\geq 6$ y in 115 (55.0%) and 63 (81.8%) patients with asthma exacerbation and stable asthma, respectively (P < .001).  
*P < .001 for between-group comparisons.

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FeNO was the only parameter that differed between patients with and without HRV, being significantly lower in the former group \((P = .018)\). Ten controls were HRV positive, and mean (SD) FeNO of those with and without HRV were 38.6 (17.9) ppb and 82.2 (61.3) ppb, respectively \((P < .001)\). Table 4 summarizes the relationship between age and different respiratory pathogens in patients with asthma exacerbation. Patients with asthma exacerbation caused by respiratory viruses were younger than those without identifiable viral infections \((P < .05)\). This finding was attributed mainly to RSV \((P < .005)\) and influenza A and HMPV infections \((P < .05\) for both). Age did not differ between the case and control groups with infections by other organisms, including HRV, or with coinfections.

Figure 1 illustrates the seasonal pattern of HRV, which was found in patients throughout the study period \((\geq 10\%)\) and peaked in winter \((November-December)\) of 2007 to 2008. Similarly, 28.6% of controls had HRV in autumn-winter \((September-October)\), but none of them were HRV positive in spring-summer \((March-August)\). The low positive rates for other respiratory pathogens in our subjects preclude our analysis of their seasonality patterns.

Table 2—Detection of Different Viral and Bacterial Pathogens in Subjects

| Individual organism                        | Asthma Exacerbation \((n = 206)\) | Stable Asthma \((n = 77)\) | \(P\) Value |
|------------------------------------------|----------------------------------|-----------------------------|-------------|
| Rhinovirus                                | 54 (26.2)                        | 10 (13.0)                   | .027*       |
| Human metapneumovirus                     | 12 (5.8)                         | 2 (2.6)                     | .364        |
| Influenza A virus                         | 16 (7.8)                         | 4 (5.2)                     | .624        |
| Influenza B virus                         | 3 (1.5)                          | 0                           | .565        |
| Parainfluenza viruses                     | 14 (6.8)                         | 2 (2.6)                     | .250        |

Table 3—Details of Asthma Exacerbations in Relation to Rhinovirus Infection

|                              | Rhinovirus | No Rhinovirus | \(P\) Value* |
|------------------------------|------------|---------------|--------------|
| Age, y                       | 7.3 (3.8)  | 7.7 (4.1)     | .462         |
| Duration of hospitalization, d | 3.4 (1.4)  | 3.7 (2.0)     | .349         |
| Duration of fever, d         | 0.40 (0.63)| 0.53 (0.93)   | .248         |
| Maximum temperature, °C      | 38.5 (0.6)| 38.7 (0.7)    | .266         |
| Vital signs                  |            |               |              |
| Minimum SaO₂, %              | 93.8 (2.8) | 94.3 (2.3)    | .333         |
| Maximum pulse rate, per min  | 139 (20)   | 135 (22)      | .181         |
| Maximum respiratory rate, per min | 34 (9)    | 34 (9)        | .645         |
| Systolic blood pressure, mm Hg | 108 (15)  | 110 (15)      | .426         |
| Diastolic blood pressure, mm Hg | 70 (11)   | 69 (10)       | .486         |

| Laboratory results            |            |               |              |
|-------------------------------|------------|---------------|--------------|
| FeNO, ppb                     | 31.7 (20.3)| 62.4 (44.8)   | .018         |
| FEV₁, % predicted             | 68.1 (28.1)| 74.3 (20.4)   | .569         |
| FVC, % predicted              | 70.7 (27.1)| 84.0 (33.4)   | .251         |
| FEV₁ to FVC ratio             | 0.82 (0.11)| 0.81 (0.27)   | .800         |
| PEF, L/min                    | 156 (57)   | 201 (51)      | .104         |

Results expressed in mean (SD). See Table 1 for expansion of abbreviations.

*Insufficient respiratory specimens were obtained from three patients.
\(\ast\) Analyzed by \(\chi^2\) (with Yates correction) or Fisher exact test as appropriate.
\(\ast\) Odds ratios (95% CI) for asthma exacerbation were: 2.38 (1.09-5.32) for rhinovirus, 2.85 (1.54-5.30) for any virus, and 2.77 (1.51-5.11) for any pathogen.

**Discussion**

Our group performed multiplex nested PCR assays on 475 children hospitalized for acute RTIs from 2005 to 2006. Respiratory pathogens were detected in 47% of these patients, and HRV peaked in winter and early spring. These multiplex nested PCR assays were specific and 100- to 1,000-fold more sensitive than conventional methods in detecting the viruses. Our assays detected \(\leq 10\) nucleic acid copies for all viruses (except enteroviruses), which were comparable to those reported in widely quoted high-throughput multiplex PCR assays. Specifically, our nested PCR was able to detect one cDNA copy of HRV. The present study used the same method, except for *Legionella pneumophila* being replaced by HBov, to investigate the infective causes of asthma exacerbation in Hong Kong children. HRV infections also peaked in winter of 2007/2008 in children with asthma exacerbation (Fig 1). Our detection rate \((51\%)\) was similar to previous local studies but lower than those published in white populations. It is uncertain whether our low HRV detection rate was due to limitations of the PCR technique, which is less likely in view of our previously noted *in vitro* results, or a genuinely low incidence of HRV infection in Hong Kong children. Further studies in other Asian populations are needed to confirm our findings.

HRV infection was associated with asthma exacerbation in the children, which is consistent with...
B. On the other hand, HRV infection was detected in 13% of our subjects with stable asthma who did not experience any symptom or sign of disease exacerbation. In a longitudinal study of healthy children, 20.6% of all HRV infections were asymptomatic. Future studies should delineate the pathogenic linkage between HRV and worsened asthma.

During the past few years, there has been impressive advance in our understanding of the interactions between HRV and host immunity. HRV infects human cells via ligation with its major group receptor intercellular adhesion molecule 1. Infected respiratory epithelial cells, and possibly macrophages, produce a variety of proinflammatory cytokines, chemokines, and leukotrienes. These mediators in turn attract different inflammatory cells to the airway, resulting in worsened immunopathology and increased bronchial hyperresponsiveness observed in patients with asthma. In addition, airway epithelium from patients with asthma is deficient in mounting adequate antiviral responses to HRV.

HBoV was detected in 5.0% of 1,906 local children hospitalized for acute RTIs, and seasonal distribution was noted from September to February. Despite this, the detection of HBoV in 2.4% of cases and 2.6% of controls was not associated with asthma exacerbation in the present study. This finding might be explained by our exclusion of infants and young children, who were at increased risk of HBoV infection.

Three-fifths of adults with asthma exacerbation had *M pneumoniae* and/or *C pneumoniae*, and telithromycin was shown to be a useful treatment in these patients. *M pneumoniae* was detected in more than half of patients with asthma, and also in upper airway secretions from 11% of patients with chronic stable asthma. Hahn claimed oral macrolides to be efficacious for patients with acute asthma. On the other hand, Cunningham et al failed to show any relation between *M pneumoniae* and childhood asthma exacerbation. *M pneumoniae* and *C pneumoniae* were detected only in 2.4% of our children with asthma exacerbations, which was similar to that observed in patients with stable asthma. Our findings do not support atypical bacteria to be important pathogens for asthma exacerbations in children or the usefulness of macrolides in treating these patients.

The major limitation of this project relates to its study power. The number of controls was much lower than that of our recruited cases, mainly because case-control matching within 1 week was not possible on many occasions when stable and especially younger patients also complained of nonspecific upper respiratory symptoms (e.g., rhinorrhea, blocked nose, sore throat) during change of weather. Our sample size had a power of 97% for detecting any difference in the detection of any virus between cases and controls.

### Table 4—The Relationship Between Different Respiratory Pathogens and Age Distributions of 206 Evaluable Patients With Asthma Exacerbations

| Individual organism                  | Infection (y) | No Infection (y) |
|--------------------------------------|---------------|------------------|
| Rhinovirus                           | 7.3 (3.8)     | 7.7 (4.1)        |
| Human metapneumovirus                | 5.8 (2.8)*    | 7.7 (4.1)        |
| Influenza A virus                    | 6.0 (3.0)*    | 7.7 (4.1)        |
| Parainfluenza viruses types 1-4      | 7.7 (4.9)     | 7.6 (4.0)        |
| Respiratory syncytial virus          | 4.5 (2.1)*    | 7.7 (4.1)        |
| Bocavirus                            | 7.4 (2.8)     | 7.6 (4.1)        |
| Adenovirus                           | 6.2 (3.8)     | 7.6 (4.1)        |
| Human coronaviruses OC43             | 8.3 (4.3)     | 7.6 (4.1)        |
| Presence of any virus                | 6.9 (3.5)*    | 8.3 (4.4)        |
| *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* | 6.3 (4.0)     | 7.6 (4.1)        |
| Presence of any pathogen             | 6.9 (3.5)*    | 8.4 (4.4)        |
| Coinfection by two or more pathogens | 6.5 (4.1)     | 7.7 (4.0)        |

Results expressed in mean (SD) and only included data for pathogens that were detected in five or more patients.

*P*, .05 for between-group comparisons.

*P*, .005 for between-group comparisons.

*P*, .01 for between-group comparisons.

Published data about the importance of HRV in white populations. In the Childhood Origins of Asthma birth cohort, a total of 259 children were followed prospectively from birth to 6 years of age. HRV-associated wheezing in years 1 and 3 were the strongest predictor for asthma diagnosis at the age of 6 years. Nearly 90% of children who wheezed with HRV in year 3 subsequently developed asthma. Two other studies found HRV to be the most important microbiological risk factors for asthma diagnosis and disease exacerbation. More recently, Miller et al reported that childhood asthma exacerbations were associated with the novel group C of HRV rather than the two previously known phylogenetic groups A and
but had a marginal power of 70% for HRV infection (GraphPad StatMate; San Diego, CA) and <50% for the detection of other respiratory pathogens because of their rarity. Thus, larger studies are needed to delineate the possible association between asthma exacerbation and RTIs by these organisms. The lack of standardization on the methodology of HRV detection would also pose a problem. A recent study revealed that HRVs consist of >100 distinct serotypes.35 In view of this degree of phylogenetic heterogeneity, the PCR primers designed for our multiplex assays were not able to detect all HRVs. Despite the use of sensitive multiplex assays as discussed previously, our molecular approach for detecting viruses would miss some HRVs that were not covered by our PCR primers. Future studies need to adopt multiple PCR primers that specifically target as many HRV serotypes as possible. Another weakness is that NPA samples were collected from 236 (83.4%) subjects, whereas nasal swabs were collected from the remaining subjects. As we previously reported, the overall sensitivity of detecting influenza, parainfluenza, RSV, and adenovirus in NPA was higher than that obtained by nasal swabs in local children.36 On the other hand, we did not have relevant data for HRV. Although more than 80% of subjects had NPA samples, it is possible that we might have missed some organisms in those with only nasal swabs. We also observed that only 30% of patients hospitalized for asthma exacerbation had successfully performed FeNO measurement according to guideline (Table 1).18 As patients with severe bronchospasm were probably too breathless for the procedure, only patients with milder attacks would contribute to FeNO readings in this group. In addition, a substantial proportion of these patients were treated with systemic corticosteroids prior to FeNO. These reasons explain the lower FeNO in these patients when compared with patients with stable asthma.

In conclusion, respiratory viruses and atypical bacteria are detected in more than half of Hong Kong children with asthma exacerbation. HRV infection is the most important risk factor for asthma exacerbation in these patients. Nonetheless, none of these pathogens is associated with severity of asthma exacerbation.

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Mr To: contributed to performing clinical and virologic investigations.
Mr Yeung: contributed to performing clinical and virologic investigations.
Mr Y. S. Wong: contributed to performing clinical and virologic investigations.

Dr G. W. K. Wong: contributed to subject recruitment and manuscript preparation.
Dr Chan: contributed to designing and supervising virologic investigations and participated in manuscript preparation.

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