

ORIGINAL ARTICLE

Viral and bacterial infection in acute asthma and chronic obstructive pulmonary disease increases the risk of readmission

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ABSTRACT

Background and objective: Infection is as an important trigger for acute asthma and chronic obstructive pulmonary disease (COPD). The aim of this article was to determine the prevalence and impact of virus and bacterial infections in acute asthma and COPD.

Methods: Subjects were recruited, within 24 h of hospital admission for acute exacerbations of asthma and COPD. Nose/throat swabs and sputum samples were collected and examined by multiplex polymerase chain reaction for respiratory viruses and cultured for bacteria. The primary outcomes were length of stay (LOS) and readmission to hospital within 60 days.

Results: A total of 199 subjects were recruited (96 had asthma and 103 COPD) for 235 events (36 re-presented). A virus was detected in 79 subjects (40%), bacteria in 41 (21%), and of these, 18 had both. Rhinovirus A was the most frequently isolated virus. A multivariate analysis was performed to control for confounders. It found that detection of a virus, a virus and bacteria, forced expiratory volume in 1 s (FEV₁) and a diagnosis of COPD were all independent predictors of prolonged LOS, while risk of readmission within 60 days was increased with virus infection alone, virus and bacterial infection, lower FEV₁ and current smoking.

Conclusions: Virus infection, especially in the presence of chronic bacterial infection, is an important determinant of more severe acute exacerbations in both asthma and COPD, and patients with co-infections are more likely to be readmitted to hospital following their exacerbation.

Key words: asthma, chronic obstructive pulmonary disease, infection and inflammation, respiratory infection, viral infection.

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; LOS, length of stay; RV, rhinovirus.

SUMMARY AT A GLANCE

A virus infection with rhinovirus A has been demonstrated to be a likely trigger of acute asthma and COPD. Virus infection, in combination with a chronic bacterial infection, is an important determinant of more severe acute exacerbations and is more likely to result in hospital readmission following severe acute exacerbation.

INTRODUCTION

Acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD) remain a major clinical problem. In COPD, exacerbations are associated with a faster decline in lung function,¹ worsened quality of life² and increased mortality.³ In asthma, severe exacerbations are also associated with a more rapid decline in lung function.⁴ In the case of both COPD⁵ and asthma,⁴ recent large prospective trials have used hospitalization to identify severe exacerbations. In addition, those with the most severe exacerbations of COPD are at higher risk of readmission to hospital within 60 days.⁶

Infection is recognized as an important trigger for exacerbations in both COPD and asthma. In asthma, respiratory virus infections are associated with the majority of acute exacerbations in both children⁷ and adults, and have also been shown to be associated with more severe acute disease.⁸ In COPD, the situation is complicated by chronic bacterial infection that is associated with more severe airflow obstruction and increased airway inflammation.⁹ Furthermore, the risk of acute exacerbations has been found to be heightened with the acquisition of new bacterial strains.¹⁰ As is the case with asthma, acute viral infection, particularly with rhinovirus (RV), is associated with acute exacerbations, including those leading to hospitalization.¹¹ However, it remains unclear what the impact of viral and bacterial infection, alone and together, has on both acute exacerbations and the chance of readmission in the context of disease severity in asthma and COPD.

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We determined the prevalence and nature of virus and bacterial infections over a period of 2 and half years to accurately characterize seasonal infection in adults with acute asthma and COPD. We recruited a large cohort of severe exacerbations requiring hospitalization; we then determined the impact of these infections on acute clinical severity as measured by length of stay (LOS) and the chance this will increase the frequency of future exacerbations.

METHODS

Subjects

Subjects included were over 16 years of age presenting with an acute exacerbation of asthma or COPD to the emergency department or clinic of the John Hunter Hospital, Newcastle, Australia. Subjects were given a diagnosis of COPD if they had prior documented evidence of airflow obstruction, defined as a ratio of forced expiratory volume in 1 s (FEV₁)/forced vital capacity less than 0.7 post bronchodilator when stable as defined by the Global Initiative for Chronic Obstructive Lung Disease 2011.¹² Asthma was defined as those with a consistent history and prior documented evidence within 2 years of variable airflow obstruction, with evidence of an increase in FEV₁ greater than 15% or 400 mL following bronchodilator or bronchial hyperresponsiveness on bronchial provocation testing, when stable.¹³ We classified those who did not correct their airflow obstruction as (ratio >0.7, post bronchodilator) as COPD. The study was approved by both the Hunter Area Health Service and the University of Newcastle Research Ethics Committees, and written informed consent was obtained from all participants prior to study entry.

Sample molecular processing

Spontaneous sputum was obtained, sputum plugs were separated and 0.1 mL was suspended in 0.9 mL Buffer RLT (Qiagen, Melbourne, Victoria, Australia) and stored at -80°C. Nasal and throat swab buds were immersed in 1 mL of Buffer RLT and stored at -80°C. Total RNA was then extracted and purified using the RNeasy Mini Kit (Qiagen) and stored at -80°C. RNA was then reverse transcribed to total cDNA, using random primers and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Sputum was cultured and bacteria identified using standardized techniques, briefly. The sputum was collected from subjects in a sterile container and transported to the laboratory within 2 h of expectoration. Smears were made from the purulent material and Gram stains performed. The remaining sample was then subjected to liquefaction and dilution. Cultures of these diluted samples were made on blood agar, chocolate agar and MacConkey agar. The plates were incubated at 37°C in an incubator in the atmosphere of 10% CO₂. After overnight incubation, the plates were transferred immediately to the Microbiology department where predominant organisms were studied by standard techniques. Optochin-sensitive organisms were studied for further confirmation of

Streptococcus pneumoniae. All pathogenic organisms were subjected to antibiotic sensitivity testing.¹⁴

Real-time virus polymerase chain reaction

Induced sputum, nasal and throat swab total cDNA was assayed by real-time polymerase chain reaction for RV, enterovirus, non-severe acute respiratory syndrome coronavirus, human metapneumovirus, respiratory syncytial virus types A and B and influenza virus types A and B RNA transcripts.

Real-time polymerase chain reaction used the fluorogenic 5' nuclease ('TaqMan') methodology. Each reaction mixture contained 5 µL cDNA, relevant primers and probe and a commercial polymerase solution (RealMasterMix Probe ROX; 5PRIME, Gaithersburg, MD, USA). All fluorogenic probes incorporated the 5' reporter dye 6-carboxyfluorescein and the 3' quencher dye 6-carboxy-tetramethyl-rhodamine. Each sample was subject to amplification with the following parameters: 2 min at 95°C to activate the HotMaster Taq DNA polymerase and 40 cycles of 95°C for 15 s followed by 1 min at 60°C (ABI 7500 cycler; Applied Biosystems, Foster City, CA, USA). Only samples yielding a Ct value of ≤36 and displaying a sigmoidal amplification curve were considered positive. Positive control virus cDNA was included in each virus assay.

Human RV sequencing

One-Step RT-PCR (Qiagen) was carried out on 33 of the RV-positive samples using a primer pair that amplified a fragment of 549 bp spanning the hyper-variable part of the 5' non-coding region, the entire VP4 gene and the 5' terminus of the VP2 gene.¹⁵ Amplified fragments were sequenced bidirectionally with BigDye Terminator Sequencing Kit (Applied Biosystems) on a 3730 DNA Analyzer (Applied Biosystems). Trace files were reviewed using Chromas Lite (Technelysium Pty Ltd, Queensland, Australia) and bidirectional sequences were compared using EMBOSS Pairwise Alignment Algorithms (EMBL-EBI, Cambridge, UK). Finally, all 33 RV-positive consensus sequences, along with eight⁸ published sequences, were aligned using ClustalW2 (EMBL-EBI) and phylogenetic analyses performed using MEGA 4.0.¹⁶

Statistical analyses

The summary data are presented as means and standard deviations of the mean. Differences between the groups at first admission were analysed using Student's *t*-test. To determine what variables were associated with the outcomes LOS and readmission, multiple logistic regression analysis was performed. All data were analysed using STATA version 11 (STATA corp, College Station, TX, USA).

RESULTS

There were 292 subjects approached to participate, 44 declined, 248 were screened, 49 were excluded as adequate documentation was not available. This

Table 1 Demographic data for patient population

	Asthma	COPD	P-value
Number	96	103	NA
Acute episodes	114	121	NA
Age, year (SD)	47.2 (19.6)	70.3 (11.4)	<0.001
Gender, M:F	30:66	50:53	0.8
Acute FEV ₁ , % predicted, mean (SD)	53.0 (14.4)	36.6 (14.8)	<0.001
ACQ6, mean (SD)	3.6 (1.5)	NA	NA
ICS dose, BDP eq mean (SD)	1853 (968)	1911 (882)	0.8
Smoking, never:current:ex	34:21:41	5:23:75	<0.01
Influenza vaccination, <i>n</i> (%)	56 (58)	51 (50)	0.3
LOS, days mean (SD)	3.4 (2.9)	4.5 (2.9)	<0.01
[†] LOS >4 days, <i>n</i> (%) acute events	31 (27%)	63 (52%)	<0.001
[†] Readmit in 60 days, <i>n</i> (%) acute events	11 (10%)	28 (23%)	<0.01
[†] Pathogens; virus only, <i>n</i> (%) acute events	37 (32%)	27 (22%)	0.4
Bacteria only, <i>n</i> (%) acute events	6 (5%)	20 (17%)	0.02
Both, <i>n</i> (%) acute events	8 (7%)	11 (9%)	0.3

[†]Pearson's chi-square test.

ACQ, Asthma Control Questionnaire; BDP, beclomethasone dipropionate; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; LOS, length of stay; NA, not applicable; SD, standard deviation.

resulted in 199 subjects that were recruited to participate, 32 re-presented within the time frame of the study and were recruited again (four of which presented a third time), resulting in 235 acute episodes between March 2007 and September 2009 (Table 1).

There were 96 subjects with asthma and 103 with COPD. Details of subjects are outlined in Table 1; those with COPD were older, had lower lung function at presentation, used higher doses of inhaled corticosteroids and were more likely to be current smokers. Those with COPD also had a longer LOS and were more likely to be readmitted to hospital within 60 days. Both groups had similar rates of influenza vaccination at 50–56%.

Virus infection

A virus was detected during 83 acute events (35%), in 79 subjects, with nine subjects having more than one virus. Of these positive samples, the most frequently isolated virus was RV, 35, (40%), of which the RV species were RV-A,¹⁷ RV-B⁵ and RV-C.⁶ Two were untypeable due to insufficient RNA. The other viruses detected were influenza,⁶ respiratory syncytial virus,¹⁸ coronavirus,¹² enterovirus⁹ and human metapneumovirus.⁸ Virus prevalence and distribution over the 36 months according to season are described in Figure 1. RV was the most frequently isolated virus in summer, autumn and winter; coronavirus was particularly evident in spring, while influenza and respiratory syncytial virus were found only in autumn and winter. Repeat analysis, 6–10 weeks following admission, was done for 71; only three were positive, two for respiratory syncytial virus and one RV.

A phylogenetic tree depicting the relationship between known RV serotypes and the RV positives from our study is shown in Figure 2.

Bacterial infection

Bacteria were isolated in 45 acute events (19%), from 41 subjects with 4 subjects having more than one

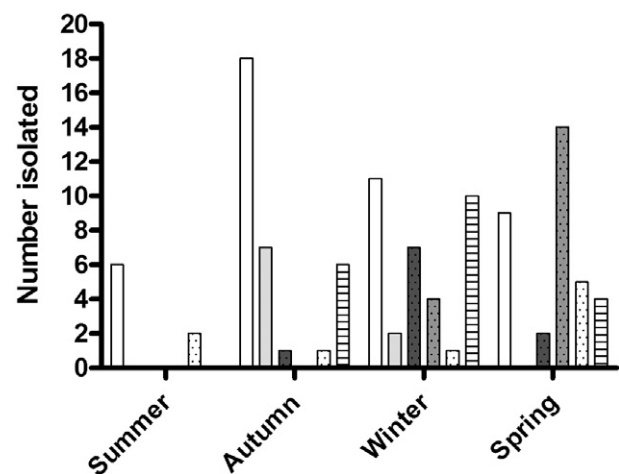


Figure 1 Seasonal distribution of virus. Number of respiratory viruses detected per season between March 2007 and September 2009. Autumn = March–May, Winter = June–August, Spring = September–November and Summer = December–February. CoV, non-severe acute respiratory syndrome coronavirus; EV, enterovirus; FLU, influenza virus (A and B), hMPV, human metapneumovirus; RSV, respiratory syncytial virus (A and B); RV, rhinovirus. □, RV; □, EV; ■, FLU; ■, CoV; □, hMPV; □, RSV.

bacteria isolated (Fig. 3); the most prevalent was *Haemophilus influenzae*, followed by *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *S. pneumoniae* and *Staphylococcus aureus*. Of these, there were 18 subjects who had both a bacteria and virus detected acutely. In contrast to viruses isolated, 34 of 78 had bacteria again isolated on culture 6 weeks after their admission; 18 *H. influenzae*, 3 *M. catarrhalis*, 6 *P. aeruginosa*, 1 *S. pneumoniae*, 1 *Stentrophomonas maltophilia* and 1 *S. aureus*. In addition, three more subjects were isolated with bacteria that had not been present acutely, three *H. influenzae* and two *M. catarrhalis*.

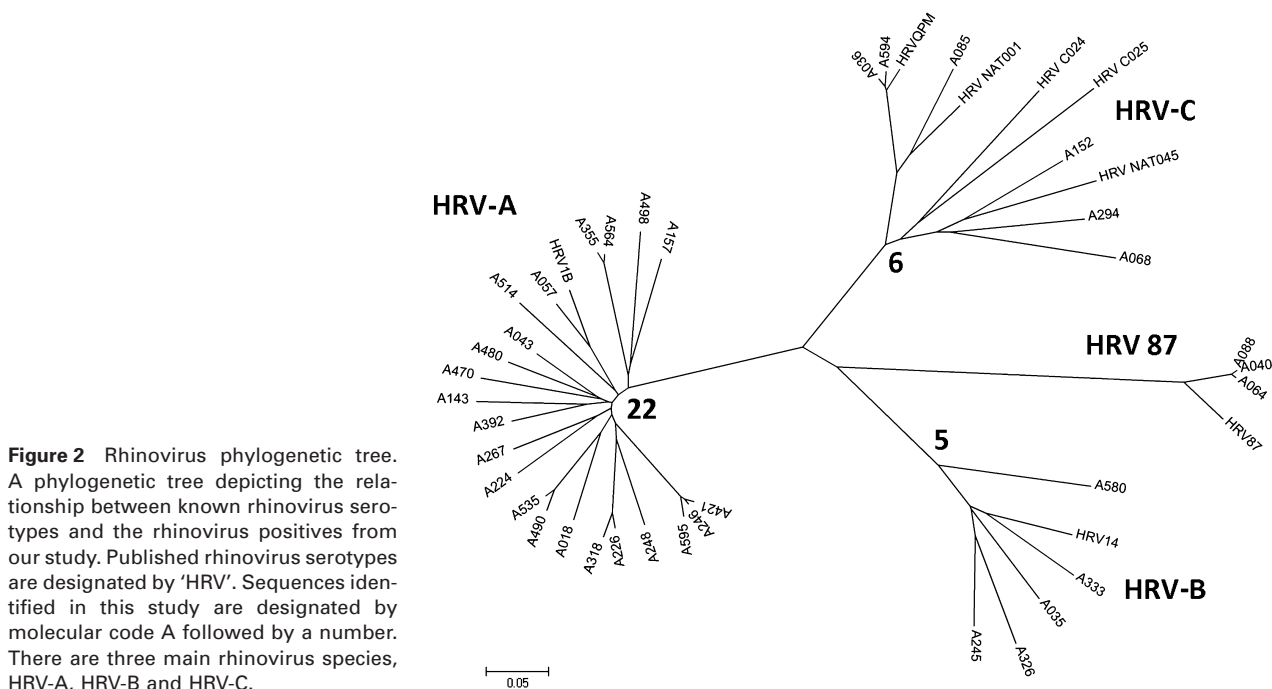


Figure 2 Rhinovirus phylogenetic tree. A phylogenetic tree depicting the relationship between known rhinovirus serotypes and the rhinovirus positives from our study. Published rhinovirus serotypes are designated by 'HRV'. Sequences identified in this study are designated by molecular code A followed by a number. There are three main rhinovirus species, HRV-A, HRV-B and HRV-C.

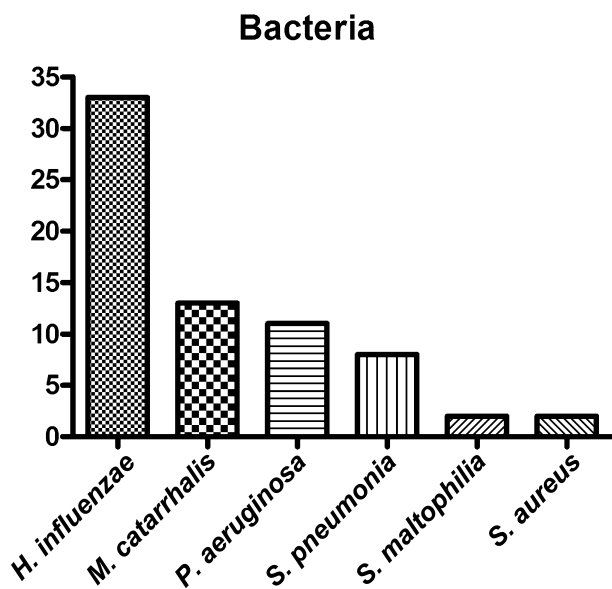


Figure 3 Bacteria isolates. Number of bacteria isolates detected between March 2007 and September 2009 for selected prominent bacteria. *Haemophilus influenzae* followed by *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Stenotrophomonas maltophilia*.

Clinical impact of infection

Univariate analyses were carried out for each virus infection, but there were no associations between type of virus infection or clinical severity as measured by FEV₁, LOS or chance of readmission (data not shown). A univariate analysis (Table 2) demonstrated that compared with those with no virus infection, those with virus infection had no difference in acute FEV₁ but did have a longer LOS and were more likely

to be readmitted. Those with bacterial infection had a lower FEV₁ at presentation, a longer LOS and were more likely to be readmitted. Those with both virus and bacterial infection had a lower FEV₁, a longer LOS and again were more likely to be readmitted to hospital. At review 6 weeks following their admission, spirometry was again measured. Spirometry was available for 167 individuals 6 months prior to their exacerbation, when that individual self-reported to have been stable. Those with virus infection had a greater decline in lung function at 6 weeks compared with those without a virus having been isolated. This was not seen in those who had bacteria isolated alone, but was seen in those who had both virus and bacteria isolated acutely.

A multivariate logistic regression model was then constructed to determine what factors independently predicted LOS and the odds of readmission. To analyse LOS, subjects were divided into those admitted for more than or less than 4 days (taken as it was the median LOS for the group); independent predictors of longer LOS were virus infection, although not virus and bacterial infection or bacterial infection alone, male gender, lower FEV₁ at presentation and a diagnosis of COPD (Table 3).

The factors associated with readmission within 60 days were then examined (Table 4). In this case, neither virus alone nor bacteria alone predicted readmission; however, readmission was more likely in those with both virus and bacterial infection, and in those with lower FEV₁ at presentation.

DISCUSSION

In hospitalized adults with acute asthma and COPD, infections were detected in 109/235 acute episodes;

Table 2 Univariate for each virus/bacterial combination

	Virus Yes (<i>n</i> = 83)	Virus No (<i>n</i> = 152)	<i>t</i> -test <i>P</i> -value
FEV ₁ at presentation (% predicted), mean (SD)	43 (19.4)	45 (18.6)	0.3
ACQ6, mean (SD)	3.4 (1.5)	3.8 (1.5)	0.2
LOS, days mean (SD)	5 (2.5)	3.4 (2.5)	<0.001
Readmission, <i>n</i>	22	17	0.003
FEV ₁ at recovery % change from pre-exacerbation, mean (SD)	−4.8% (1.7)	−2.1% (2.3)	0.02
	Bacteria Yes (<i>n</i> = 45)	Bacteria No (<i>n</i> = 190)	
FEV ₁ at presentation (% predicted), mean (SD)	34 (19.3)	47 (19.6)	<0.001
ACQ6, mean (SD)	3.7 (1.5)	3.1 (1.5)	0.4
LOS, days mean (SD)	5.6 (2.8)	3.6 (2.5)	0.001
Readmission, <i>n</i>	22	21	<0.001
FEV ₁ at recovery % change from pre-exacerbation, mean (SD)	−3.6% (1.5)	−2.4% (1.9)	0.4
	Virus and bacteria Yes (<i>n</i> = 19)	Virus and bacteria No (<i>n</i> = 216)	
FEV ₁ at presentation (% predicted), mean (SD)	32 (19)	46 (14)	0.001
ACQ6, mean (SD)	3.6 (1.5)	3.6 (1.2)	0.9
LOS, days mean (SD)	6.9 (3.1)	3.7 (2.6)	<0.001
Readmission, <i>n</i>	16	2	<0.001
FEV ₁ at recovery % change from pre-exacerbation, mean (SD)	−5.9% (1.7)	−1.9% (2.3)	0.01

The number given, *n*, refers to acute events.

ACQ, Asthma Control Questionnaire; FEV₁, forced expiratory volume in 1 s; LOS, length of stay; SD, standard deviation.

Table 3 Multivariate logistic regression for independent factors determining length of stay

Variables	Odds ratio	95% CI	<i>z</i>	<i>P</i> > <i>z</i>
Virus	3.0	1.16, 7.6	2.28	0.02
Bacteria	0.8	0.2, 3.1	−0.31	0.9
Virus and bacteria	5.5	0.4, 69	1.31	0.3
Age	0.98	0.95, 1.01	−1.5	0.1
Gender	3.2	1.3, 7.9	2.5	0.01
Smoking status	1.8	1.0, 3.1	2.06	0.8
ICS BDP/day	0.99	0.99, 1.0	−1.4	0.04
FEV ₁ (% predicted)	0.96	0.94, 0.99	−2.5	0.03
COPD	4.6	1.7, 12.5	3.02	0.003

BDP, beclomethasone dipropionate; CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid.

Table 4 Multivariate logistic regression for independent factors determining readmission

Variables	Odds ratio	95% CI	<i>z</i>	<i>P</i> > <i>z</i>
Virus	0.9	0.2, 4.7	−0.1	0.9
Bacteria	2.3	0.4, 12.9	0.3	0.3
Virus and bacteria	32.2	1.1, 93	2.02	0.04
Age	1.0	0.51, 1.02	0.08	0.9
Gender	0.6	0.1, 2.8	−0.7	0.6
Current smoker	1.3	0.5, 3.2	0.3	0.6
ICS BDP/day	1.0	0.99, 1.0	0.4	0.7
FEV ₁ (%predicted)	0.91	0.85, 0.97	0.004	0.004
COPD	2.7	0.5, 10.8	0.2	0.2

BDP, beclomethasone dipropionate; CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroid.

35% were viral, 19% bacterial, while 8% had both. The most prevalent virus over the 2 years was RV, belonging to the RV-A clade. In terms of the acute impact of infection, as measured by LOS, virus infection, lower FEV₁ at presentation, current smokers, male gender and a diagnosis of COPD were all independent predictors. In terms of increasing the risk of hospital readmission in 60 days, acute virus and bacterial infection and lower FEV₁ at presentation were independent predictors.

Severity of pre-existing disease, as measured by FEV₁, has previously been shown to be an important

predictor of exacerbation and acute severity in asthma¹⁹ and COPD,¹⁸ and our results again confirm this, with acute FEV₁ an independent predictor for both LOS and readmission. Recently, this was confirmed in a large prospective trial of COPD, but importantly, the other factor that was found to be independently associated with exacerbation risk was the occurrence of exacerbations themselves.⁵ In the context of acute infection, our results emphasize that there is an important interaction between acute infection and severity of airflow obstruction. In addition, we have decided to divide subjects into those

with wholly reversible airways disease (asthma) and those with incomplete reversibility, COPD. We acknowledge that such a description may be a departure from traditional diagnostic paradigms for airway disease that focus on a mixture of history, exposure and airway physiology. We have remained essentially true to this in terms of asthma, with subjects when stable needing to demonstrate variable airflow obstruction; but within this group, we chose not to exclude smokers. Nonetheless, the group we have defined as asthma were younger, were less likely to have smoked and had smoked less compared with those classified COPD. In contrast, those with COPD did not necessarily have a history of smoking, emphasizing that smoking-induced fixed obstructive airway disease does not have to be solely synonymous with COPD. In this way, we feel we have been able to examine the effects of fixed airway disease, for which COPD stands, and found that independent of smoking status, fixed airway disease is an important predictor in multivariate analysis for both LOS and readmission.

If specific antiviral strategies are to be considered in treating acute exacerbations, the pattern of these infections needs to be clearly defined. We assessed acute exacerbations over 2 and half years in a large cohort with acute COPD and asthma. Winter was associated with spikes of infection with respiratory syncytial virus and influenza, the latter occurring despite rates of greater than 50% vaccination. This level of vaccination is probably inadequate,²⁰ and of the patients, 4/6 with influenza have not been vaccinated and one had received the vaccine within 3 weeks of admission. In both years, there was a peak of non-severe acute respiratory syndrome coronavirus in spring. RV was the most prevalent virus through all seasons, with the exception of spring. We did not demonstrate that one virus was associated with more severe clinical disease. Although the individual numbers of viruses isolated was not large, this study may lack the power to discern small differences between the viruses; it seems unlikely that such differences in severity are clinically meaningful.

The high annual prevalence of RV in our cohort confirms its importance in acute asthma and COPD, and for the first time in adults, we sequenced the responsible RV strains. The recent discovery of a new species of RV-C²¹ has led to renewed interest as to whether specific RV species may be associated with more severe clinical disease. In young children with acute asthma, there does appear to be such an association with one large US study demonstrating RV-C followed by RV-A was associated with severe exacerbations.²² This was particularly marked in a Western Australia study, where the majority of children with acute asthma had RV-C and it again was associated with more severe disease.¹⁷ Our findings in adults do not support the assertion that the majority of exacerbations are due to RV-C, with RV-A clearly playing a prominent role. The differences with our findings could be explained in several ways. RV-C may particularly affect children, with a possible tropism for asthma and atopic disease.¹⁷ Any variation in RV-induced exacerbations may also reflect the strains

circulating in the community, as we did not include non-asthmatic/COPD adults or children, this remains unclear. Alternatively, RV-C may be more difficult to identify in adults, who usually present later with exacerbations compared with children, and shed less virus for shorter periods of time. Nonetheless, RV-A strains are known experimentally to cause a significant increase in symptoms in adults with asthma²³ and COPD,²⁴ and our results confirm them to be important pathogens associated with exacerbations.

We also demonstrated that co-infection with viruses and bacteria were associated with an increased risk of readmission. It has been shown previously in a smaller prospective study in subjects with COPD that co-infection led to longer LOS and worse lung function.²⁵ *In vitro* RV is capable of promoting bacterial infection through disruption of the epithelium²⁶ and chronic bacterial infection with *H. influenzae* potentiates the inflammatory response to RV.²⁷ RV has also been shown to increase the *H. influenzae* burden during acute COPD.²⁸ Those with chronic bacterial infection therefore appear to be particularly susceptible to the effects of virus infection, while our results suggest this is relevant in both asthma and COPD. How this occurs and how co-infection worsens underlying chronic airway disease warrant further investigation. As yet, no specific interventions have looked at minimizing the effect of RV virus infection in adults with asthma and COPD, although these exist.²⁹ Macrolide antibiotics are also an interesting potential therapeutic, they reduce exacerbation risk in COPD³⁰ and may reduce the impact of RV infection at least *in vitro*.³¹ However, it is also worthy to consider that uncomplicated and inexpensive infection control procedures such as hand-washing and wearing surgical masks are potentially very effective at limiting virus transmission and can be employed by patients and their families at home.³²

In conclusion, our results confirm the important independent role of virus infection along with viral/bacterial co-infection as independent predictors of acute severity in COPD and asthma and for the first time demonstrate that those with co-infection are also more likely to be readmitted to hospital following their exacerbation. Interventions are needed that minimize the effect of virus infection on both asthma and COPD, especially in the context of chronic bacterial infection. Given the marked clinical effect these infections have on patients with COPD and asthma, specific interventions need to be assessed especially in those with more severe disease prone to exacerbations.

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