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# Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease<sup>☆</sup>

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Accepted 14 November 2005

Available online 10 January 2006

## KEYWORDS

Metapneumovirus;  
Lung disease;  
Obstructive lung  
disease;  
Paramyxoviridae  
infection

**Summary** *Objective:* Respiratory viruses are a common trigger for exacerbations of chronic obstructive pulmonary disease (COPD). Human metapneumovirus (hMPV) is a paramyxovirus associated with respiratory tract infections and wheezing. Our aim was to determine whether hMPV was associated with exacerbations of COPD.

*Methods:* The study was designed as an observational cohort study carried out in a 944-bed urban teaching hospital located in New Haven, Connecticut. Between December 2002 and May 2003, patients hospitalized due to an exacerbation of COPD were identified. Nasopharyngeal specimens obtained from these patients were tested for human metapneumovirus by RT–PCR and for respiratory syncytial virus, influenza A and B, parainfluenza-1, -2, and -3 and adenovirus by a cytospin-enhanced direct immunofluorescence assay and/or viral culture.

*Results:* Fifty individuals met enrollment criteria and hMPV was identified in 6 (12%), respiratory syncytial virus in 4 (8%), influenza A in 2 (4%) and parainfluenza type 3 in 1 (2%) patients. Both A and B hMPV genotypes were identified in patients hospitalized due to exacerbations of COPD.

<sup>☆</sup> Presented in part at the 42nd Infectious Diseases Society of America Annual Meeting, Boston, MA, September 30 to October 3, 2004; Abstract 985.

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**Conclusion:** hMPV was frequently identified in patients hospitalized due to an exacerbation of COPD. Further studies are necessary to determine the epidemiology and the impact of hMPV in COPD patients.

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## Introduction

In the United States, over 14 million people have chronic obstructive pulmonary disease (COPD) and COPD is the fourth leading cause of death.<sup>1</sup> Much of the morbidity, mortality and economic impact of COPD is due to acute exacerbations.<sup>2,3</sup> Exacerbations of COPD have been associated with bacterial and viral respiratory tract infections as well as exposure to airborne pollutants.<sup>4</sup> Two studies by Seemungal et al. have noted that one-third to 50% of exacerbations of COPD are associated with respiratory virus infections.<sup>5,6</sup>

In 2001 van den Hoogen et al. reported the discovery of a novel paramyxovirus, human metapneumovirus (hMPV).<sup>7</sup> hMPV has since been identified globally.<sup>8</sup> Similar to other paramyxoviruses, such as respiratory syncytial virus (RSV) and parainfluenza, hMPV appears to have a seasonal distribution and has been primarily identified during the winter and early spring in North America.<sup>9–11</sup> hMPV has been isolated from both children and adults with upper and lower respiratory tract infections and has been associated with the acute onset of wheezing.<sup>9,10,12</sup> However, the full spectrum of disease caused by hMPV remains to be determined. We sought to determine the frequency of hMPV in adults hospitalized due to exacerbations of COPD.

## Methods

### Patient enrollment and viral testing

From December 13, 2002 through May 6, 2003 all patients hospitalized at Yale-New Haven Hospital (a 944-bed urban teaching hospital providing primary and tertiary level care located in New Haven, Connecticut) with an admission diagnosis of an acute exacerbation of COPD were identified. Nasopharyngeal specimens were obtained at the discretion of the clinician caring for the patient and transported to the laboratory in MicroTest M4-RT medium (Remel, Lenexa, KS) within 1 h. Specimens obtained overnight were held at 4 °C for up to 8 h. Otherwise, specimens were processed by the laboratory on receipt. hMPV testing was

performed using a reverse transcriptase–polymerase chain reaction assay (RT–PCR) with primers specific for the hMPV F gene as previously described.<sup>11</sup> RSV, influenza A and B, parainfluenza-1, -2 and -3 and adenovirus were identified by a cytospin-enhanced direct immunofluorescence assay (DFA).<sup>13</sup> Nasopharyngeal specimens required a minimum of 25 columnar epithelial cells to be adequate for DFA testing. Viral cultures were performed, if requested by the clinician, using primary rhesus monkey kidney, A549 and MRC-5 cell cultures, according to published methods.<sup>13</sup> These techniques are unable to identify either rhinovirus or coronaviruses. Patients were excluded from the analysis if a nasopharyngeal specimen was unavailable, not obtained within the first 5 days of hospitalization or inadequate for DFA testing. Medical records were reviewed to obtain information regarding the patients' clinical course of their acute illness and past medical history. If a patient was hospitalized more than once for an acute exacerbation of COPD during the study period, only the first acute exacerbation hospitalization was included since the duration of hMPV shedding has not been well described.

hMPV genotype was determined by phylogenetic analysis of a 120-nucleotide segment of hMPV F gene corresponding to nucleotides 3304 through 3423 of hMPV NL/1/00 (GenBank accession number [AF371337](#)). A phylogenetic tree was derived by the maximum likelihood method using DNAML (PHYLIP, version 3.6, Dr. Joseph Felsenstein, University of Washington) with default settings and a bootstrap dataset containing 500 permutations produced by BOOTSEQ (PHYLIP). An extended majority-rule consensus tree was produced using CONSENSE (PHYLIP).

### Statistical analysis

The *t*-test for independent samples and Fisher's exact test were used to compare continuous and categorical variables, respectively. All comparisons were two-tailed and performed using SPSS version 13.0 (SPSS, Chicago, IL). *P* values less than or equal to 0.05 were considered significant. Ninety-five percent confidence intervals (CI) were calculated using the Clopper–Pearson method

and StatXact-4 software (Cytel Software Corp., Cambridge, MA).<sup>14</sup>

The Human Investigations Committee at the Yale University School of Medicine approved this research.

## Results

Between December 13, 2002 and May 6, 2003, 102 patients were hospitalized 105 times for an exacerbation of COPD and 50 (49%) patients met enrollment criteria. Twenty-four (24%) patients were excluded since they did not have a nasopharyngeal specimen obtained during the first five hospital days, 18 (18%) patients were excluded since no remaining nasopharyngeal specimen was available for hMPV testing and 10 (10%) patients were excluded since their nasopharyngeal specimen was deemed inadequate for DFA testing.

hMPV was identified in 6 of 50 (12%, CI 5–24%) patients, RSV in 4 (8%, CI 2–19%), influenza A in 2 (4%, CI 1–14%) and parainfluenza type 3 in 1 (2%, CI 0–11%) patient. The remaining 37 (74%) were negative for RSV, influenza, parainfluenza, adenovirus and hMPV. All 10 specimens deemed

inadequate for DFA testing were negative for hMPV by RT–PCR. No patient was identified with more than one virus.

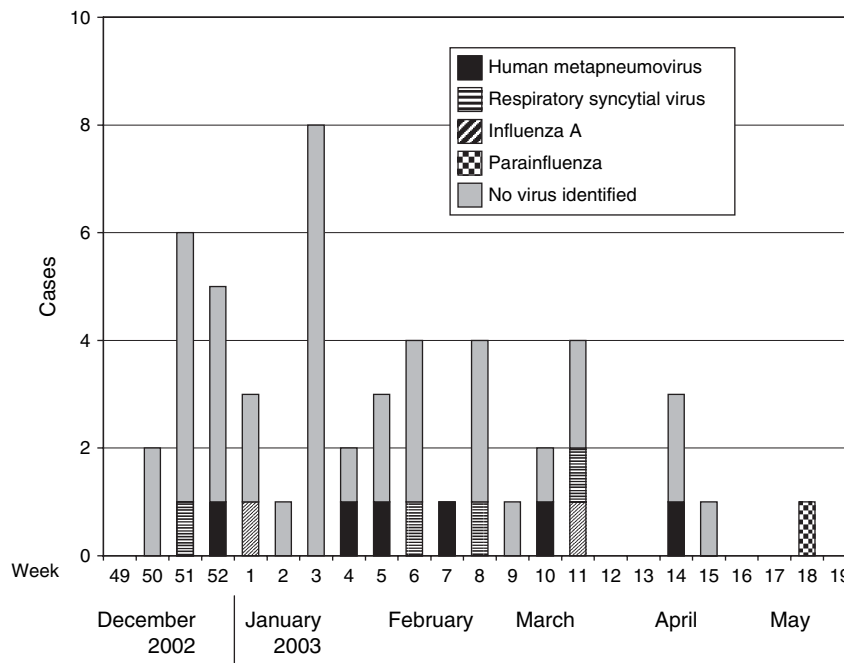
No significant differences in the demographic or clinical characteristics were noted between the hospitalized acute exacerbation of COPD patients identified with or without evidence for hMPV infection. However, patients with hMPV associated acute exacerbations were more frequently febrile ( $>38^{\circ}\text{C}$ ) during their hospitalization (Table 1). All of the patients with hMPV were hospitalized between December 25, 2002 and April 3, 2003 (Fig. 1) and noted a change in the character of their cough, sputum production and level of dyspnea, consistent with an accepted definition of an acute exacerbation of COPD.<sup>15</sup> These patients all received supportive care, broad spectrum antibiotics, systemic steroids and supplemental oxygen during their hospitalization. Three of the six patients with hMPV had evidence for a lower respiratory tract infection by the presence of a new infiltrate on chest radiograph (patients 2, 5 and 6). A urine Legionella antigen test was performed for one patient (patient 6) and was negative. Gram stain and culture was performed on sputa available from three patients (patients 1, 2 and 3) and failed

**Table 1** Demographic and clinical characteristics of COPD exacerbation patients

	hMPV associated exacerbation of COPD ( <i>n</i> = 6)	Non-hMPV associated exacerbation of COPD ( <i>n</i> = 44)	<i>P</i>
Median age in years (SD)	64 (13)	70 (18)	n.s.
Male (%)	4 (40)	17 (67)	0.381
Caucasian (%)	3 (50)	28 (64)	n.s.
Home oxygen use	3 (50)	14 (37)	n.s.
Current tobacco use	0 (0)	14 (40)	0.143
Pre-hospital steroid use			
No steroids	1 (17)	21 (55)	0.375
Inhaled steroids	3 (50)	10 (26)	0.339
Systemic steroids	2 (33)	11 (29)	n.s.
Fever ( $\geq 38^{\circ}\text{C}$ )	4 (67)	9 (24)	0.053
Leukocytosis ( $\geq 12,000$ )	3 (50)	10 (27)	0.345
ICU admission	2 (33)	9 (24)	n.s.
Mechanical ventilation	2 (33)	9 (24)	n.s.
Infiltrate on chest X-ray	2 (33)	5 (13)	0.238
Median length of stay in days (range, SD)	4 (3–16, 5)	5 (1–51, 10)	0.458
Exacerbation of COPD type <sup>15,a</sup>			
Type 1	6 (100)	28 (74)	0.310
Type 2	0 (0)	3 (8)	n.s.
Type 3	0 (0)	7 (18)	n.s.

Data for some variables were not available from all enrolled patients. SD, standard deviation. n.s., not significant ( $P > 0.5$ ).

<sup>a</sup> Type 1 exacerbations were defined as the occurrence of increased dyspnea, sputum production and cough. A Type 2 exacerbation is defined as the presence of 2 of these 3 symptoms and a Type 3 exacerbation is defined as the presence of one of these 3 symptoms along with at least one of the following: upper respiratory tract signs or symptoms during the preceding 5 days, fever, increased wheezing, tachypnea, or tachycardia.<sup>15</sup>



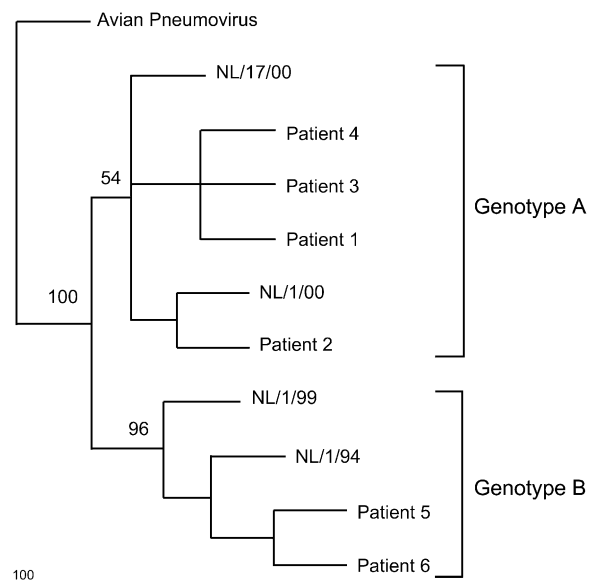
**Figure 1** Epidemic curve of exacerbation of COPD hospitalizations and associated respiratory viruses.

to reveal any potential bacterial pathogens. No patient identified with hMPV was diagnosed or suspected of having an acute bacterial respiratory infection during the period of hospitalization. Among the acute exacerbation of COPD patients without evidence for hMPV, sputum Gram stain and culture were performed for 42% and urine Legionella tests were obtained from two patients. Bacterial diagnoses of methicillin-resistant *Staphylococcus aureus* and *Haemophilus influenzae* infection were each made in one of the 44 patients.

Four of the six patients with hMPV clinically improved after the initial 48 h of hospitalization as reflected by the decreased use of supplemental oxygen. Two patients with hMPV-associated acute exacerbations of COPD required mechanical ventilation. One patient received non-invasive mask ventilation, clinically improved and was discharged after 3 days of hospital care. The other patient had a history of severe COPD (FEV<sub>1</sub> of 0.56 L/s measured 7 years prior to this admission) and required invasive mechanical ventilation for 3 days due to hypoxic, hypercapnic respiratory failure. This patient slowly improved and was discharged home after a total 16 days hospitalization. Inadequate pulmonary function testing data was available to allow reliable comparison between the hMPV and non-hMPV groups.

Phylogenetic analysis using hMPV F Gene nucleotide sequences showed that the exacerbations of COPD were associated with both recognized hMPV genotypes (Fig. 2).<sup>16</sup> Exacerbations with

the hMPV genotype A were found earlier during the winter season, between December 2002 and February 2003, while hMPV genotype B associated exacerbations were noted in March and April 2003. Comparison of hMPV A and B genotype



**Figure 2** Phylogenetic tree of hMPV isolated from COPD at Yale-New Haven Hospital, 2002–2003. Relevant bootstrap values are shown at tree branch points, where applicable. Additional sequences obtained from GenBank are as noted: Avian Pneumovirus, AF368170; NL/17/00, AY304360; NL/1/00; AF371337, NL/1/99, AY304361; NL/1/94, AY304362.

sequences revealed a total of 21 single nucleotide substitution sites within the 120 nucleotide F gene segment from the six hMPV isolates; 19 of these 21 sites were located in the third codon position. One codon contained substitutions in both the first and third position, but neither polymorphism encoded for an amino acid change. There was 97.5% deduced amino acid sequence homology between A and B genotypes for this F gene segment.

## Discussion

Respiratory viruses are responsible for a significant proportion of exacerbations of COPD and are a major cause of disability for this population.<sup>7–12</sup> Recently, other investigators have identified hMPV in 5.5% of patients experiencing an exacerbation of COPD when no other microbial pathogen could be identified.<sup>17</sup> However, a recently published study found no evidence for hMPV infection among 194 acute respiratory illnesses experienced by 96 patients with COPD during a nearly 4-year study period.<sup>17,18</sup> Both studies used RT–PCR to identify hMPV. It remains unknown whether temporal and/or geographic differences in the circulation of hMPV may have led to the differences in these findings.

This study was performed during the winter and early spring when hMPV is typically detected.<sup>9–11</sup> The incidence of both RSV and influenza A also typically peak during the winter and are on the decline by early spring in North America. Thus, the frequency of exacerbations associated with hMPV, RSV and influenza were likely to be greatest during the study period and were found similar to rates observed by other investigators.<sup>5,6,19</sup> We were unable to successfully determine if patients had or had not received influenza vaccination during the 2002–2003 season. It remains unclear whether hMPV significantly affects patients with COPD during other seasons in temperate climates. We did not identify any specific demographic or clinical characteristic that distinguishes hMPV from non-hMPV acute exacerbations of COPD, however, our study was of limited power to identify such characteristics.

RT–PCR was used to identify hMPV in this study as it has become the standard diagnostic test for hMPV.<sup>20</sup> hMPV is difficult to culture and DFA testing is currently not available commercially.<sup>21</sup> RSV, influenza, parainfluenza and adenovirus were detected by cytospin-enhanced DFA and tissue culture; methods routinely employed in clinical virology laboratories. In our laboratory, cytospin

enhanced DFA has been shown to have a sensitivity similar to conventional cell culture for the identification of influenza, parainfluenza and RSV, and is comparable to real-time RT–PCR for the detection of influenza A.<sup>13,22</sup>

The hMPV infections observed in our patients likely represented re-infection since it has been shown that most persons are seropositive for hMPV by 5–10 years of age.<sup>7,23</sup> As with RSV and parainfluenza, the duration and degree of protective immunity may wane with time. It has been shown that hMPV isolates segregate into two distinct genotypes by phylogenetic analysis.<sup>10,11,16</sup> Therefore, it is possible that infection with one hMPV genotype may not confer complete protective immunity against other hMPV strains. Previous reports have described patients infected with genetically distinct hMPV strains during sequential years, however, this issue remains unresolved.<sup>16,24–26</sup>

It has been suggested that co-infection of hMPV and RSV may potentially explain some of the variance in the severity of RSV illness in children and that hMPV co-infection may play a role in the pathogenesis of the severe acute respiratory syndrome (SARS).<sup>27–29</sup> In our study, we did not identify any patients with an exacerbation of COPD co-infected with RSV and hMPV despite focusing on patients with severe illness resulting in hospitalization.

Our investigation only included hospitalized patients. A significant proportion of exacerbations of COPD either do not require hospitalization or are not brought to medical attention.<sup>6</sup> It has been shown that many adult patients with hMPV infection experience a mild to moderate respiratory illness that often leads to medical evaluation but infrequently results in hospitalization.<sup>9</sup> It is therefore possible that hMPV may have greater impact on the quality of life and healthcare utilization for patients with COPD than is suggested by our findings.

The duration of hMPV carriage, the frequency of asymptomatic carriage and the optimal period for diagnostic testing has not been well characterized. Our study was based on review of medical records, documented by many and often different healthcare providers. Due to this significant inter-observer variability, we did not quantitate the duration between onset of illness and diagnostic testing. Concern has been raised regarding the duration of viral nucleic acid carriage and hence the specificity of PCR based diagnostic testing in patients with COPD.<sup>30</sup> However, hMPV is only rarely identified in patients lacking signs or symptoms of an acute respiratory illnesses. Williams, et al. identified hMPV in only 1 (1.2%) of 86 children without



evidence for a respiratory tract infection.<sup>10</sup> Furthermore, the recent study by Beckham et al. failed to identify any hMPV infections in a group of COPD patients who experienced an acute respiratory illness.<sup>18</sup> This suggests that the identification of hMPV from a patient experiencing an exacerbation of COPD more likely represents the etiology for the exacerbation rather than an unrelated epiphenomena.

## Conclusions

In this study, we have shown that hMPV was frequently associated with exacerbations of COPD resulting in hospitalization. Prospective population-based studies are necessary to further define the impact of hMPV on patients with COPD, the epidemiology of hMPV, methods to prevent hMPV infection and its potential role as a co-pathogen with other respiratory pathogens. If hMPV is shown to have significant impact on patients with COPD as is suggested by this study, the development of a hMPV vaccine may merit consideration.

## Acknowledgements

We are indebted to Eugene D. Shapiro for his critical review of the manuscript and insightful comments. We are also indebted to the staff of the Clinical Virology Laboratory, Linda Selander for her assistance in Medical Records and Bobbie Welch for assisting with patient surveillance. This work was supported by The Patrick and Catherine Weldon Donaghue Medical Research Foundation (J.S.K.) and the William Wirt Winchester Foundation (R.A.M.). This work was also supported in part by the Yale Children's Clinical Research Center grant M01-RR06022 (J.S.K.), General Clinical Research Centers Program, National Center for Research Resources, National Institutes of Health and by National Institutes of Health grants T32 AI07210-20 (F.E.) and F32 AI055151 (F.E.). The authors do not claim conflict of interest related to the material presented herein.

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