Human Metapneumovirus Infection in Adults with Community-Acquired Pneumonia and Exacerbation of Chronic Obstructive Pulmonary Disease

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(See the editorial commentary by Mandell on pages 490–7 and the article by van Gageldonk-Lafeber et al. on pages 503–6)

We tested nasopharyngeal aspirate specimens by real-time polymerase chain reaction assays and paired serum samples by enzyme-linked immunosorbent assays. Acute human metapneumovirus infections were identified in 6 (4.1%) of 145 adult patients who presented to the emergency department for pneumonia or acute exacerbation of chronic obstructive pulmonary disease during 2 winter/spring seasons in Quebec, Canada.

Human metapneumovirus (hMPV) is a newly described member of the Paramyxoviridae family that is composed of 2 major groups (groups A and B) [1]. Along with human respiratory syncytial virus (hRSV) and influenza viruses, hMPV is one of the leading causes of acute respiratory tract infections (ARTIs), such as bronchiolitis and pneumonia, in children aged <3 years during the winter/spring seasons [2, 3]. Although hMPV has been associated with influenza-like illnesses, bronchitis, and pneumonias in adults [4–6], there are limited data on the incidence of this viral infection in well-defined cohorts of adults. Therefore, we examined the role of hMPV infection in adults with community-acquired pneumonia (CAP) and exacerbation of chronic obstructive pulmonary disease (COPD).

PATIENTS, MATERIALS, AND METHODS

The study was conducted during the periods of 17 January 2002 through 6 May 2003 (hereafter “year 1”) and 6 January 2003 through 6 May 2004 (hereafter “year 2”) at 3 university-affiliated hospitals. During year 1, two groups of patients were enrolled: (1) subjects with COPD aged ≥40 years who presented to the emergency department with exacerbation of illness (including subjects with and subjects without pneumonia), and (2) subjects without COPD aged ≥18 years who were admitted to the hospital with a diagnosis of CAP. In both cases, patients were excluded if they presented ≥7 days after the onset of symptoms. During year 2, only subjects with exacerbation of COPD (with or without pneumonia) were recruited. The study was approved by the ethics committees of all participating health care centers.

After informed consent was obtained, a nasopharyngeal aspirate (NPA) specimen was collected for RT-PCR studies using a flexible catheter (14 French), and a questionnaire was completed for all participants. In addition, a pair of blood samples was collected at a 3–4-week interval for serological testing. All NPA specimens were first tested using a multiplex real-time PCR assay for influenza viruses A and B, hRSV, and hMPV. The multiplex assay for the first 3 viruses has been described elsewhere [7]. Primers for the hMPV polymerase gene that were included in the new multiplex PCR assay were as follows: 5′-GTT GCC ATA GAT AAT CCT GTT A-3′ (forward) and 5′-CAA ATT ACT ACT AA
CCA ATT GCT TAC CCA-3′ (reverse). The lower limit of detection (per PCR reaction) for the multiplex assay was 50 copies for influenza A and B, 100 copies for hRSV, and 250 copies for hMPV using transcribed plasmids. Each specimen that was positive for hMPV by the multiplex real-time PCR was also tested with another real-time PCR for the hMPV nucleoprotein gene amplifying a 109-bp fragment. The primers used for the latter PCR assay were as follows: 5′-GCA TTT CCG AGA ACA ACA C-3′ (forward) and 5′-GCT TAG CA/GT AA/TG AAA TTT CTC C-3′ (reverse). The genotype of the hMPV strains was determined by sequencing a larger region (750-bp) of the nucleoprotein gene, followed by alignment with corresponding sequences in GenBank.

Detection of hMPV antibodies was performed by ELISA with recombinant nucleoproteins from hMPV groups A and B [8]. This ELISA was previously validated using a Western immunoblot assay. All acute-phase and convalescent-phase serum samples were first screened at a 1:20 dilution. For pairs of serum samples that showed increasing optical density values of ≥0.1, serial 2-fold dilutions were tested to determine the exact hMPV serological titer. Serological studies for influenza and hRSV were performed using a standard complement-fixation methodology. Seroconversion was defined as a ≥4-fold increase in viral titers between acute-phase and convalescent-phase serum samples.

RESULTS

Virological findings. A total of 98 patients were enrolled in year 1 of the study, including 64 subjects with COPD who had acute exacerbation of their illness and 34 subjects with CAP who did not have COPD, whereas 47 patients (all of whom had COPD exacerbations) were recruited in year 2. All patients with COPD were hospitalized (in accordance with inclusion criteria), compared with 80.8% of subjects with COPD exacerbations. Two (2.0%) of the subjects in year 1 tested positive for hMPV by the multiplex real-time PCR, whereas 4 (8.5%) tested positive in year 2 (table 1). Only 1 (16.7%) of the 6 hMPV-infected subjects tested positive for another viral pathogen (influenza virus A) by multiplex real-time PCR. All specimens that tested positive for the hMPV polymerase gene (part of the multiplex assay) were subsequently confirmed by a second real-time PCR assay for the hMPV nucleoprotein gene. Sequencing of the nucleoprotein gene revealed that 5 hMPV strains belonged to group A and 1 belonged to group B.

In year 1, a total of 83 (84.7%) of the 98 subjects had detectable hMPV antibodies in their acute-phase serum samples, compared with 39 (83.0%) of the 47 patients in year 2. A ≥4-fold increase in hMPV antibody levels was found for 4 (66.7%) of 6 PCR-positive patients (2 in year 1 and 2 in year 2) (table 1). In addition, a 2-fold increase in hMPV antibody levels was noted for 1 PCR-positive patient, and high titers (1:1280) in the acute-phase serum sample were observed for another. As a comparison, the reciprocal of the mean antibody titer of 20 randomly selected adults was found to be 69 using the same assay. No episodes of seroconversion were noted for patients with negative PCR results. There was a very good correlation between patients’ hMPV titers when determined by ELISAs with the recombinant nucleoprotein from hMPV group A or B as the antigen (table 1). Thus, the overall incidence of hMPV infection in our patient group, as determined by real-time PCR and/or serologic testing, was 6 (4.1%) of 145 patients over the entire study. Of note, influenza virus A, influenza virus B, and hRSV were detected in 6.2%, 0%, and 9% of patients, respectively, by the use of multiplex PCR and serological testing. Clinical findings in hMPV-infected patients. Selected characteristics of the 6 hMPV-infected subjects are presented in table 1. The mean age of these patients was 64.2 years (range, 39–78 years). Four patients had an history of COPD (2 of whom also had inactive lung cancer), 1 patient had asthma, and 1 had underlying congestive heart failure. All hMPV-positive patients were identified during the month of April for both years. There was a mean of 4.8 days (range, 2–6 days) between the onset of symptoms and the collection of the NPA specimen for hMPV testing. Only 1 (16.6%) of the hMPV-positive patients had another microorganism identified in the sputum culture (Streptococcus pneumoniae, in addition to influenza virus A and hMPV, in his NPA specimen).

Four patients (66.6%) had a final diagnosis of pneumonia (2 of these patients had underlying COPD), whereas acute exacerbation of COPD (without pneumonia) was diagnosed in the other 2 patients. All hMPV-positive patients were hospitalized (mean duration of hospitalization, 10 days; range, 4–23 days). None of these subjects were admitted to the intensive care unit, and none died during hospitalization. All 6 patients initially presented to the emergency department with cough and sore throat, 5 had fever (mean oral temperature, 38.3°C; range, 37.1°C–39.6°C), 5 had dyspnea, 4 had nasal congestion and/or rhinorrhea, 3 had purulent sputum, 3 had wheezing, and only 2 had myalgia. All patients had decreased oxygen saturation at the time of consultation (mean, 88.2% at room air; range, 82%–93%), and all received treatment with broad-spectrum antibiotics (mainly respiratory quinolones or third-generation cephalosporins plus macrolides).

DISCUSSION

Our study demonstrates that hMPV is associated with a significant number of cases of CAP and COPD exacerbations in adults during the early spring season. hMPV circulation predominating in March and April has also been reported in Canadian children during 2 consecutive years (2001–2003) [2, 9]. The incidence of hMPV infections in our adult population...
Table 1. Clinical and laboratory findings for human metapneumovirus (hMPV)–infected patients with pneumonia or exacerbation of chronic obstructive pulmonary disease (COPD).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Date that NPA specimen was obtained</th>
<th>Diagnosis</th>
<th>Other underlying disease</th>
<th>Bacterial pathogen</th>
<th>Duration of hospitalization, days</th>
<th>hMPV genotype</th>
<th>Multiplex RT-PCR findingb</th>
<th>hMPV RT-PCR findingc</th>
<th>hMPV titersa</th>
<th>N-A protein</th>
<th>N-B protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Timed from NPA specimen to onset of symptoms, days</td>
<td>Diagnosis</td>
<td>Other underlying disease</td>
<td>Bacterial pathogen</td>
<td>Duration of hospitalization, days</td>
<td>hMPV genotype</td>
<td>Multiplex RT-PCR findingb</td>
<td>hMPV RT-PCR findingc</td>
<td>hMPV titersa</td>
<td>N-A protein</td>
<td>N-B protein</td>
</tr>
<tr>
<td>86</td>
<td>64</td>
<td>F</td>
<td>1 April 2003</td>
<td>4</td>
<td>Pneumonia (left side)</td>
<td>CHF</td>
<td>5</td>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>1:640</td>
<td>1:2560</td>
<td>4</td>
</tr>
<tr>
<td>97</td>
<td>39</td>
<td>M</td>
<td>10 April 2003</td>
<td>2</td>
<td>Pneumonia (right side)</td>
<td>Asthma</td>
<td>6</td>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>1:320</td>
<td>1:1280</td>
<td>4</td>
</tr>
<tr>
<td>127</td>
<td>74</td>
<td>M</td>
<td>2 April 2004</td>
<td>5</td>
<td>COPD exacerbation</td>
<td>Lung cancer</td>
<td>23</td>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>1:160</td>
<td>1:1280</td>
<td>8</td>
</tr>
<tr>
<td>138</td>
<td>60</td>
<td>M</td>
<td>20 April 2004</td>
<td>4</td>
<td>COPD exacerbation</td>
<td>None</td>
<td>4</td>
<td>B</td>
<td>Positive</td>
<td>Positive</td>
<td>1:160</td>
<td>1:2560</td>
<td>16</td>
</tr>
<tr>
<td>140</td>
<td>78</td>
<td>F</td>
<td>23 April 2004</td>
<td>6</td>
<td>Pneumonia and COPD</td>
<td>Diabetes</td>
<td>17</td>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>1:640</td>
<td>1:1280</td>
<td>2</td>
</tr>
<tr>
<td>144</td>
<td>72</td>
<td>M</td>
<td>29 April 2004</td>
<td>6</td>
<td>Pneumonia and COPD</td>
<td>Lung cancer</td>
<td>5</td>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>1:1280</td>
<td>1:1280</td>
<td>1</td>
</tr>
</tbody>
</table>

NOTE. CHF, cardiac heart failure; NPA, nasopharyngeal aspirate; S. pneumoniae, Streptococcus pneumoniae.

a As determined by ELISA using the recombinant nucleoprotein from hMPV genotype A (N-A) or genotype B (N-B).
b Real-time PCR for hMPV (polymerase gene), human respiratory syncytial virus (fusion gene), influenza virus A (matrix gene), and influenza virus B (matrix gene).
c Real-time PCR for hMPV (nucleoprotein gene).
varied from 2.0% in 2002–2003 to 8.5% in 2003–2004, for an overall rate of positivity of 4.1%. hMPV infections occurred half as frequently as hRSV infections (9%) but were almost as prevalent as influenza A cases (6.2%). Using RT-PCR, we previously showed that the incidence of hMPV infections was 5.3% in children during year 1 of this study [9]. Our data also indicate that hMPV infections in the elderly population (in particular, in patients with COPD) may lead to severe lower respiratory tract infections requiring hospitalization. Indeed, all hMPV-infected subjects in our study had severe hypoxemia (mean oxygen saturation in room air, 88.2%) and prolonged hospitalization (mean duration, 10 days).

hMPV is now a well-established cause of ARTI (mostly bronchiolitis) in young children [2, 3, 10]. In addition, hMPV infections have been associated with asthma exacerbations in some studies [11, 12]. However, the pathologic role and the incidence of hMPV in ARTIs in adults has been much less studied. In a retrospective study, our group previously reported the detection of hMPV in respiratory samples obtained from elderly subjects who had been hospitalized for pneumonia and bronchitis [4]. However, no incidence data were reported in that study. In another investigation by Stockton et al. [5], hMPV was identified in 2.2% of patients of all ages with flulike illnesses who were seen by general practitioners. A larger prospective study that evaluated the role of hMPV in adults was recently reported by Falsye et al. [6]. The authors found an incidence of 4.5% in adults with various respiratory clinical syndromes. However, the study population consisted of multiple cohorts of young and elderly adults, with or without underlying disease, who came from the community or from long-term care facilities.

We found a similar rate of hMPV infection (4.1%) in a more selected and defined adult population consisting mainly of subjects with COPD who presented to an emergency department. In contrast to previous data [6], most of our patients had fever, which made their illness indistinguishable from that caused by bacterial pathogens. Indeed, all hMPV-infected patients in our study received prolonged courses of broad-spectrum antibiotic therapy, despite the fact that only 1 of 6 subjects was coinfected with a bacterial pathogen (S. pneumoniae). The absence of other viral or bacterial pathogens coupled with evidence of seroconversion in most patients suggest that hMPV was the likely cause of respiratory exacerbation in our study. Of note, Falsye et al. [6] found evidence of hMPV infection by serological testing in 4.1% of asymptomatic adults, raising questions about the causative role of hMPV in that population. However, that study was based on seroconversion over a 5–6-month period, complicating the interpretation of the results.

With use of a newly described ELISA serological assay based on recombinant hMPV nucleoproteins [8], we found that ~85% of our adult population had preexisting hMPV antibodies, which is close to the seroprevalence rate found using another assay [1]. On the other hand, it is likely that these antibodies are not fully protective, as seen in the symptomatic hMPV-infected patients in our study. A loss of protective antibodies or reinfection by a different hMPV genotype could account for hMPV disease in adults. We found that real-time PCR with NPA samples was more sensitive than serological testing of paired serum samples for identification of hMPV infection. However, a prolonged delay (6 days) between the onset of symptoms and obtainment of the first blood sample could explain the absence of seroconversion in the 2 PCR-positive patients with high hMPV titers in their acute-phase serum samples.

In conclusion, we found that hMPV is a relatively important viral pathogen that can lead to severe lower respiratory tract infections (especially in elderly patients with COPD) during the early spring season. Larger controlled studies are still needed to evaluate the impact of this newly described viral pathogen in various adult populations.

Acknowledgments

G.B. is a senior researcher scholar of the “Le Fonds de la Recherche en Santé du Québec,” and M.E.H. is a PhD scholar from the Canadian Institutes of Health Research training program in respiratory health.

Financial support. Research grants from the Canadian Institutes of Health Research (CIHR-MOP-62789; to G.B.) and “Le Fonds de la Recherche en Santé du Québec” (FRSQ-Respiratory Health Network; to G.B).

Potential conflicts of interest. All authors: no conflicts.

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