MAJOR ARTICLE

Detection of Severe Human Metapneumovirus Infection by Real-Time Polymerase Chain Reaction and Histopathological Assessment

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Background. Infections with common respiratory tract viruses can cause high mortality, especially in immunocompromised hosts, but the impact of human metapneumovirus (hMPV) in this setting was previously unknown.

Methods. We evaluated consecutive bronchoalveolar lavage and bronchial wash fluid samples from 688 patients—72% were immunocompromised and were predominantly lung transplant recipients—for hMPV by use of quantitative real-time polymerase chain reaction (PCR), and positive results were correlated with clinical outcome and results of viral cultures, in situ hybridization, and lung histopathological assessment.

Results. Six cases of hMPV infection were identified, and they had a similar frequency and occurred in a similar age range as other paramyxoviral infections. Four of 6 infections occurred in immunocompromised patients. Infection was confirmed by in situ hybridization for the viral nucleocapsid gene. Histopathological assessment of lung tissue samples showed acute and organizing injury, and smudge cell formation was distinct from findings in infections with other paramyxoviruses. Each patient with high titers of hMPV exhibited a complicated clinical course requiring prolonged hospitalization.

Conclusions. Our results provide in situ evidence of hMPV infection in humans and suggest that hMPV is a cause of clinically severe lower respiratory tract infection that can be detected during bronchoscopy by use of real-time PCR and routine histopathological assessment.

Lower respiratory tract infections can cause high mortality, especially in immunocompromised hosts. In this population, infection with common respiratory tract viruses can cause mortality of 40%, so prompt diagnosis by isolation of a causative agent is critical to prevent morbidity and mortality and to avoid unnecessary procedures, tests, and treatments that carry significant risk [1–3]. In this setting, flexible fiber-optic bronchoscopy to obtain bronchoalveolar lavage (BAL) fluid and transbronchial biopsy are frequently used to identify the cause of infection [4]. Despite these aggressive approaches, an infectious agent cannot be identified in 20%–65% of patients [3–5]. Missed diagnoses may be due to the poor sensitivity of the diagnostic tests as well as the occurrence of emergent pathogens. Moreover, even when a pathogen is found, it may be difficult to be certain that the isolated organism is the cause of the illness.

Improvements in the diagnosis of viral infection may be achieved when innovative approaches are used to discover new viruses. In this regard, human metapneumovirus (hMPV) was newly discovered by random polymerase chain reaction (PCR) amplification of viral RNA that was isolated from children with respiratory tract illnesses [6]. After its initial isolation in the Netherlands in 2001, hMPV was identified in lower respiratory tract infections in children worldwide [7–10]. Analysis of stored nasopharyngeal swab samples indicated that hMPV may account for a significant portion of respiratory tract infections with previously unknown etiology [6, 10, 11]. The consequences of hMPV infection range from acute upper respiratory tract infection to severe bronchiolitis

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or pneumonitis, and the pattern of illness is similar to that found in respiratory syncytial virus (RSV) infection [8, 9, 11]. Illness associated with hMPV develops in a broad age range, but young children, the elderly, and immunocompromised hosts appear to be at greatest risk for severe illness [8, 9, 11]. In addition, hMPV has been isolated in 51%–55% of patients with severe acute respiratory syndrome (SARS), but its contribution to that illness remains uncertain [12, 13]. Indeed, to our knowledge, neither a pathological characterization of hMPV infection nor a correlation with clinical findings has been reported in humans. Moreover, the prevalence and other characteristics of hMPV infection in the clinical setting of patients undergoing bronchoscopy for respiratory tract infections still need to be defined.

In the present study, we therefore developed and applied quantitative real-time PCR to screen for hMPV in bronchial wash (BW) and/or BAL fluid samples. Positive results afforded us the opportunity to further define the clinical and pathological features of illness in patients infected with hMPV and to identify a subset of patients with severe illness.

PATIENTS, MATERIALS, AND METHODS

Patient characteristics. A total of 806 sequential samples were collected from patients who underwent bronchoscopy from 15 December 2002 to 1 August 2003. Samples obtained within 10 days from the same patient were excluded from analysis, so that a total of 688 fluid samples (576 BAL and 112 BW) from 688 patients were analyzed. All samples that were screened for viruses were routinely submitted to the St. Louis Children's Hospital Clinical Virology Laboratory, which serves Washington University Medical Center and acts as a referral laboratory for the St. Louis metropolitan area. Bronchoscopy was performed at St. Louis Children's Hospital and Barnes-Jewish Hospital at Washington University Medical Center as well as at affiliated community hospitals. The sample period encompassed the peak winter-spring period for detection of hMPV in nasopharyngeal swab samples [14]. The mean age of the patients was 38.6 years; 26% of the patients were <18 years old, 62% were 18–65 years old, and 12% were >65 years old. Eighty-six percent of the patients had underlying conditions, most commonly in the setting of lung transplantation (table 1). Predominantly, indications for bronchoscopy were unexplained respiratory tract symptoms or abnormalities found on chest radiographs (table 2). The cohort also included 210 patients without acute illness who underwent routine bronchoscopy for surveillance after lung transplantation or follow-up bronchoscopy after treatment for transplant rejection.

Virus detection. For the detection of several non-hMPV viruses (influenza virus, parainfluenza virus, adenovirus, RSV, herpes simplex virus, and rhinovirus), samples were examined by direct immunofluorescence assay (DIFA) and for cytopathic effect (CPE) in cell culture, as described elsewhere [15]. Cytomegalovirus (CMV) was detected by shell-vial culture [15]. Testing for hMPV on samples stored at −70°C was performed after institutional review board approval. For each sample, a 140-μL aliquot was used for the isolation of RNA by use of the QIAamp viral RNA kit (Qiagen). A 5-μL aliquot of this solution was used for the detection of virus by use of quantitative real-time PCR with the TaqMan One-Step RT-PCR kit, in accordance with the manufacturer's protocol (PE Biosystems). Primers and probes were derived from a conserved fragment of the nucleocapsid (N) gene from the NL00-1 sequence of hMPV (GenBank accession no. AF371337). Direct and reverse primers amplified nt 993–1064 of the N gene. This system specifically detects hMPV genotype A, which was predominant in the study region during the study period; genotype A accounted for 75% of all hMPV-positive samples (E.A., K.C.S., and M.J.H., unpublished data). A synthetic RNA standard for hMPV was generated from a portion of the N gene (nt 40–1172) and was cloned into the pGEM plasmid (Promega). RNA was transcribed in vitro using the T7 MEGA script kit (Ambion). The DNA template was eliminated by TRIzol extraction (Invitrogen), DNase treatment, RNeasy Mini Kit preparation (Qiagen), and final phenol extraction. The concentration of the RNA standard was determined by measurement of the optical density and was used to calculate the copy numbers of hMPV RNA. The PCR amplification cycles were as follows: initial heating at 48°C for 30 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. These conditions allowed for the detection of as few as 5 copies of the RNA standard and linear amplification of the standard from 10 to 10^6 molecules. For all hMPV-positive samples, RNA isolation and hMPV real-time PCR were repeated, and hMPV-negative samples were excluded from further analysis. In addition, all samples that were found to be positive for hMPV by real-time

Table 1. Characteristics of 688 study patients who underwent bronchoscopy and bronchoalveolar lavage.

Underlying condition	No. (%) of patients
Organ transplant	438 (64)
Lung	419 (61)
Lung and other solid organ	7 (1)
Solid organ	12 (2)
Hematologic malignancy	37 (5)
Chronic lung disease	31 (5)
HIV/AIDS	27 (4)
Other systemic disease	21 (3)
Cancer	19 (3)
Other single-organ disease	17 (2)
Autoimmune disease	12 (2)
None	47 (7)
Uncertain	46 (7)

Table 2. Indications for bronchoscopy and bronchoalveolar lavage fluid sample analysis.

Indication	No. (%) of patients
Unexplained respiratory tract symptoms/radiographic abnormality	280 (41)
Respiratory failure	100 (16)
Lung transplant	
Routine surveillance	161 (23)
Follow-up after rejection	50 (7)
Decreased pulmonary function test	30 (4)
Postoperative assessment	29 (4)
Unknown	38 (6)

PCR were tested by a second real-time PCR, which detected genomic RNA for a different gene than that used for the initial detection. The primers and probe of this second real-time PCR were designed to amplify a fragment (nt 7155–7241) of hMPV STL43-84, which was isolated in our laboratory from a nasopharyngeal swab sample [14]. All samples were also routinely processed for bacterial and fungal culture and were selectively processed for atypical pathogens (*Pneumocystis, Legionella,* and *Mycoplasma* species).

In situ hybridization. Detection of hMPV was performed using in situ hybridization, because no sensitive and reproducible anti-hMPV antibody was yet available. cDNA encoding the hMPV N gene (nt 40–1172; GenBank accession no. AF371337) was inserted into the pGEM-3Zf(+) plasmid (Promega) and was transcribed into viral genomic RNA using the T7 promoter. The antisense RNA probe template was linearized by *Ssp*I digestion, and the probe was labeled with ^{35}S using an RNA labeling kit (Promega). The transcribed RNA was purified by phenol extraction and ethanol precipitation and was used as a probe to detect hMPV N gene mRNA by in situ hybridization, as described elsewhere [16]. Because a sense control probe will also detect hMPV genomic RNA, rhesus monkey kidney LLC-MK2 cells inoculated with or without hMPV were used as positive and negative controls, respectively, for lung biopsy samples. The fidelity of the probe was also tested on LLC-MK2 cells that were cultured in α-MEM with bovine serum albumin (1 mg/mL) and trypsin (1 μg/mL) and then inoculated with or without hMPV STL43-84. Before in situ hybridization, cells were checked for syncytial formation and for reactivity with a convalescent-phase serum sample from an hMPV-infected patient (patient 6). For these experiments, cultured cells were blocked with 2% gelatin, were incubated with the convalescent-phase serum sample (1:100 dilution) for 1 h and then with fluorescein isothiocyanate–conjugated anti–human IgG (1:400 dilution; Jackson Laboratory), and finally were subjected to immunofluorescence photomicrography.

Histopathological assessment and clinical outcome. The histopathological appearance of the lung tissue samples and the clinical outcome were reviewed for each patient with detectable hMPV, in accordance with a protocol that was approved by the institutional review board of Washington University School of Medicine. Lung biopsy samples were available for study in 5 of 6 patients and were subjected to hematoxylin-eosin staining and immunostaining for adenovirus, CMV, and herpes simplex virus as described elsewhere [17]. In addition, pathologists examined lung tissue samples from 4 additional patients whose BAL fluid samples were positive for RSV, rhinovirus, or parainfluenza virus. Pathologists reviewed all lung tissue samples in a blinded manner. Clinical characteristics and outcomes were determined by a retrospective review of medical records.

RESULTS

PCR-based detection of hMPV in BW and BAL fluid samples. hMPV was detected by real-time PCR in samples from 6 patients (designated patients 1–6) at a rate that was similar to that found for other common respiratory tract viruses detected by DIFA and CPE (table 3). As was expected for an immunocompromised population, CMV was also detected at a high frequency in the samples (table 3). The detection rate for influenza virus was low, probably because a rapid screening test was available and the need for proceeding to bronchoscopy was decreased. None of the 6 patients with hMPV were coinfected with other viruses, and no hMPV was detected in the 210 asymptomatic patients who underwent routine bronchoscopy for surveillance after lung transplantation or follow-up after treatment for transplant rejection. All occurrences of hMPV infection were clustered in late winter/spring, when hMPV was prevalent in the area. hMPV was the sole organism isolated from BAL or BW fluid samples in 4 patients (patients 2, 3, 5, and 6). In 2 patients, we compared viral copy numbers in BW fluid samples with those in BAL fluid samples and found that the BW fluid samples contained higher viral copy numbers than the BAL fluid samples. For all patients with severe lower respiratory tract illness (patients 1, 2, 3, 5, and 6), the results of routine bacterial and fungal cultures were negative for sig-

Table 3. Viral isolates from bronchoalveolar lavage fluid samples from symptomatic patients.

Virus (method for detection)	No. of samples
Cytomegalovirus (shell vial culture)	48
HSV (conventional culture)	15
Rhinovirus (conventional culture)	6
RSV (DIFA)	6
Parainfluenza (DIFA and culture)	3
Influenza B (DIFA)	4
Adenovirus (DIFA)	1
hMPV (real-time PCR)	6
Total	89

NOTE. A total of 478 samples were obtained from symptomatic patients. DIFA, direct immunofluorescence assay; hMPV, human metapneumovirus; HSV, herpes simplex virus; PCR, polymerase chain reaction; RSV, respiratory syncytial virus.

nificant pathogens. In addition, the results of the DIFA for *Pneumocystis* species (patients 1, 2, 5, and 6), PCR for *Mycoplasma* (patient 1), and culture or urine antigen testing for *Legionella* species (patients 1, 2, and 5) were negative.

Clinical course of hMPV infection. All patients presented with nonspecific acute respiratory tract symptoms that included fever, cough, dyspnea, and wheezing. Four of 6 illnesses occurred in immunocompromised patients (table 4). Patients 2 and 6 presented with acute respiratory failure that required mechanical ventilation. Chest radiographs of these patients showed diffuse infiltrates that were suggestive of acute lung injury (figure 1). Patients 1, 5, and 6 required prolonged hospitalization because of their acute respiratory tract illness. Patient 3 presented with exacerbation of chronic obstructive pulmonary disease and required mechanical ventilation. Patient 4, who had the mildest illness, coincidentally developed mild upper respiratory tract symptoms at the time of routine surveillance bronchoscopy after lung transplantation. Patients with severe illness due to hMPV infection (patients 1, 2, 5, and 6) tended to have higher levels of hMPV in BAL and BW fluid samples than did patients with mild illness, but the small number of patients was insufficient to establish a significant correlation. Additional clinical samples were available from patients 1 and 6. For patient 1, a nasopharyngeal swab sample was negative for hMPV at 1 week after the BAL fluid sample was obtained. For patient 6, tracheal aspirate and nasopharyngeal swab samples showed a low hMPV titer of 50 and 1032 copies, respectively, at 15 days after bronchoscopy. Each of these findings was compatible with partial or complete clearance of the virus as well as greater sensitivity of BAL and BW fluid samples for viral detection.

In situ detection of hMPV infection. Infection with hMPV was confirmed using immunofluorescence microscopy and in situ hybridization of samples. Here, we took advantage of an hMPV isolate (designated STL43-84) that we had obtained from a nasopharyngeal swab sample and had characterized by nucleotide sequencing [14]. This isolate was used to inoculate cultured LLC-MK2 cells that exhibited CPE and syncytial formation that are typical in paramyxoviral infection (figure 2). This system was then used as a substrate for testing an available convalescent-phase serum sample that was obtained from a patient in the present cohort. Thus, hMPV-infected LLC-MK2 cells, but not control cells, showed immunofluorescent staining with the convalescent-phase serum sample from patient 6, thereby indicating the presence of anti-hMPV antibody in this patient's serum (figure 2). In addition, we were able to demonstrate that hMPV-infected LLC-MK2 cells, but not control cells, gave a positive signal by in situ hybridization with a ^{35}S-labeled antisense probe for hMPV N gene mRNA (figure 2), thereby validating this approach for further analysis of lung biopsy samples.

Using the same antisense probe for hMPV, we next found a positive signal by in situ hybridization for hMPV mRNA in the open lung biopsy sample from patient 1 (figure 3). The signal seemed (on the basis of morphological appearance) to localize predominantly to type II alveolar epithelial cells and to have a heterogeneous and patchy distribution (figure 3). A lower but still significant signal by in situ hybridization was also found in bronchial epithelial cells (figure 3). Signals for hMPV mRNA were not detected in the few smudge cells found in the samples that were subjected to in situ hybridization. The transbronchial biopsy samples that were available for study from patients 2, 5, and 6 did not show any focal signal by in situ hybridization for hMPV.

Histopathological assessment of hMPV infection. Lung tissue samples were available from 5 of 6 patients with hMPV infection (all but patient 3). Lung tissue samples from 3 patients—from an open lung biopsy (patient 1) and 2 transbronchial biopsies (patients 2 and 5)—showed similar findings. The major finding in each case was acute and organizing lung injury; this included areas of what resembled diffuse alveolar damage with hyaline membrane formation and foci of bronchiolitis-obliterans/organizing pneumonialike reaction. Each sample included enlarged type II pneumocytes with smudged hyperchromatic nuclei that resembled the smudge cells found in adenovirus infection (figure 4). However, immunostaining for adenovirus, CMV, and herpes simplex virus was negative in all lung biopsy samples. A transbronchial biopsy sample from patient 4 showed changes after lung transplantation without evidence of lower respiratory tract infection. The small transbronchial biopsy sample from patient 6 showed only nonspecific acute and chronic inflammation. Smudge cells were not detected in the samples from the 4 patients with lower respiratory tract infection who had RSV, rhinovirus, or parainfluenza virus detected in BAL fluid samples.

Table 4. Clinical characteristics and outcomes for patients with human metapneumovirus (hMPV) infection.

Patient	Age	Sex	Date of sample(s)	Underlying condition	Clinical symptoms	Radiographic findings	Other organisms	Biopsy findings	hMPV copies	Clinical outcome
1	11 months	F	7 Feb 2003	Acute lymphocytic leukemia	Fever, hoarseness, increased work of breathing	Right upper lobe infiltrate and collapse	α-hemolytic *Streptococcus*	Organizing pneumonia, DAD, smudge cells	28496 (BALF)	41-day hospital stay[a]
2	71 years	M	19 Feb 2003	Hypertension, alcohol abuse	Cough, dyspnea	Diffuse bilateral alveolar and interstitial infiltrates	None	Organizing pneumonia, DAD, smudge cells	93620 (BWF)	Mechanical ventilation, 11-day hospital stay
3	59 years	M	20 Feb 2003	Severe COPD	Cough, dyspnea	Emphysema, no infiltrate	None	Not done	84 (BALF), 406 (BWF)	Mechanical ventilation, 30-day hospital stay
4	31 years	M	4 Mar 2003	Heart/lung transplant	Hoarseness, sore throat	Apical scarring, central bronchiectasis no change from findings on previous exam	α-hemolytic *Streptococcus, S. pneumoniae, Moraxella catarralis*	Chronic interstitial inflammation, no acute rejection	5493 (BALF)	Improved in 2–3 days
5	60 years	M	21 Mar 2003	Lung transplant	Cough, dyspnea, wheezing	Posttransplantation changes, no change from findings on previous exam	None	Organizing pneumonia, smudge cells, no acute rejection	1600 (BALF), 254261 (BWF)	15-day hospital stay
6	73 years	M	24 Apr 2003	Chronic lymphocytic leukemia	Cough, dyspnea, fever	Bilateral infiltrates	None	Acute and chronic inflammation	523591 (BALF)	Mechanical ventilation, 30-day hospital stay

NOTE. BALF, bronchoalveolar lavage fluid; BWF, bronchial wash fluid; COPD, chronic obstructive pulmonary disease; DAD, diffuse alveolar damage.

[a] After recovering from acute respiratory tract illness, the patient received induction chemotherapy during this hospitalization.

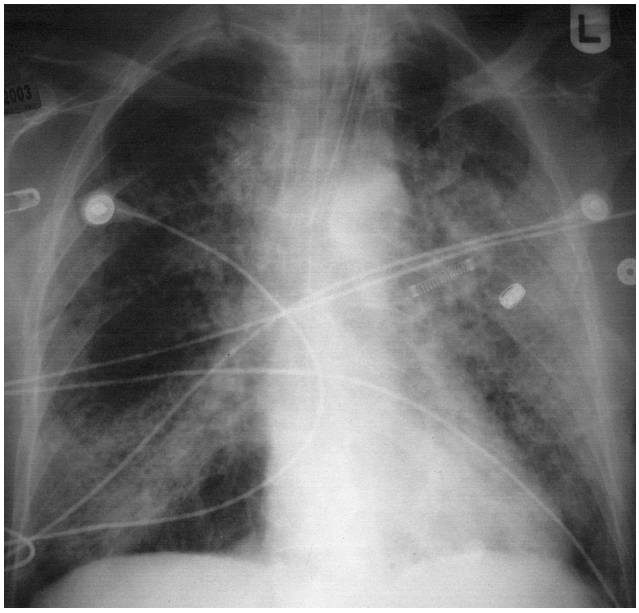

Figure 1. Radiographic evidence of severe pneumonia in human metapneumovirus (hMPV) infection. A representative chest radiograph from a patient with hMPV isolated from a bronchoalveolar lavage fluid sample (patient 2 in table 4) indicates diffuse pulmonary infiltrates with consolidation, particularly in the lingula and right lower lobe.

DISCUSSION

Since the discovery of hMPV, several studies have confirmed that the virus can be detected (generally by PCR) in clinical samples (generally nasopharyngeal swab samples) from children and adults with acute respiratory tract illness. Its occasional detection in asymptomatic patients has also been reported [9], which raises the question of whether there is sufficient specificity in this diagnostic approach. In the present study, we add several critical pieces to the diagnostic and clinical matrix for hMPV infection: (1) we detected hMPV at a frequency similar to that of other respiratory tract viruses in patients who underwent bronchoscopy for suspected respiratory tract infection; (2) we quantitatively detected hMPV by real-time PCR in bronchoscopy samples from patients with severe respiratory tract illness but not in those without symptoms or signs of respiratory tract infection; (3) we used a viral culture system to develop a specific antisense probe for hMPV and then demonstrated hMPV mRNA in the lung tissue samples; and (4) we found organizing and acute lung injury and the prominent formation of smudge cells in the lung tissue samples of these patients, thereby suggesting that this pattern may be characteristic of hMPV infection. Taken together, the detection of high copy numbers of hMPV by real-time PCR and of smudge cell formation by transbronchial biopsy can be of significant diagnostic value in defining the etiology of severe respiratory tract illness.

The pattern of the present histological findings for hMPV infection is somewhat unusual. We found that, in severe illness, hMPV is capable of causing acute pneumonia and lung injury in a pattern of diffuse alveolar damage with hyaline membrane formation that is similar to the lung injury found in other types of viral respiratory tract infections. The other member of the subfamily Pneumovirinae, RSV, which causes clinical disease that is similar to hMPV infection, can also cause acute lung injury with desquamated reactive pneumocytes, and this leads to accumulation of cellular debris within the alveoli and small airways. The presence of multinucleated giant cells (syncytial cells) with eosinophilic cytoplasmic inclusions are strongly sug-

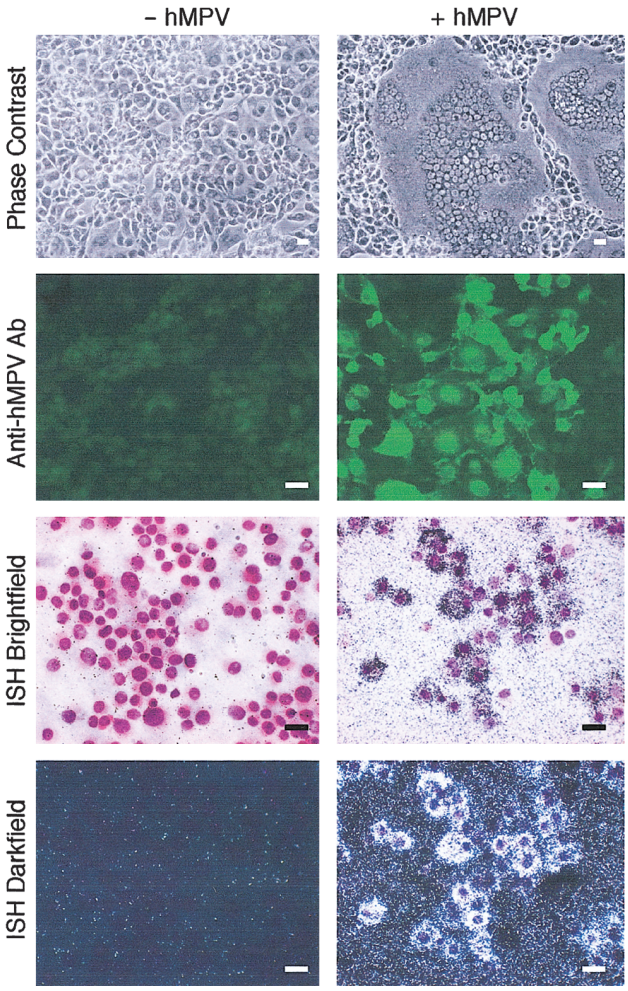

Figure 2. Detection of human metapneumovirus (hMPV) infection in cell culture. Cultured rhesus monkey kidney LLC-MK2 cells were inoculated with either hMPV (+ hMPV) or a control (− hMPV) and then were incubated at 37°C for 5 days. Cells were then subjected to phase-contrast microscopy *(first row)* at 5 days after inoculation, immunofluorescence microscopy with patient convalescent-phase serum (1:100 dilution) and fluorescein isothiocyanate–conjugated goat anti-human IgG *(second row)* at 2 days after inoculation, and in situ hybridization (ISH) with ^{35}S-labeled antisense probe for viral nucleocapsid gene mRNA under brightfield *(third row)* and darkfield microscopy *(fourth row)* at 2 days after inoculation. Scale bars, 20 μm. Ab, antibody

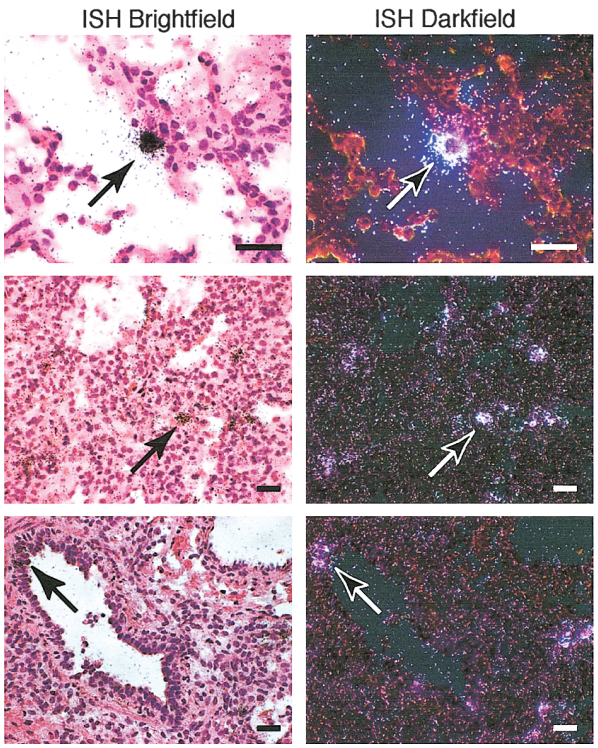

Figure 3. Detection of human metapneumovirus (hMPV) infection in lung tissue. The open lung biopsy sample from patient 1 was subjected to in situ hybridization (ISH) for hMPV mRNA and brightfield and darkfield microscopy, as described in figure 2. Arrows indicate alveolar epithelial cells *(first and second rows)* and bronchial epithelial cells *(third row)* with a positive signal above background levels. Scale bars, 20 μm.

gestive of RSV infection [18, 19], and we showed that hMPV can also cause syncytial cell formation in cell culture in vitro. By contrast, in vivo, hMPV infection appears to cause the formation of easily distinguishable smudge cells that have not been found in other paramyxoviral infections [20]. These smudge cells closely resemble the enlarged pneumocytes with darkly stained homogeneous inclusion bodies that are found in respiratory tract infection with adenovirus (a virus that causes a pattern of respiratory tract illness that is indistinguishable from that caused by hMPV). The smudge cells found in adenovirus infection have been shown to contain the virus by use of electron microscopy, in situ hybridization, and immunohistochemistry in autopsy series [21–23]. In contrast, we were able to detect hMPV mRNA in the alveolar and airway epithelial cells but not in the smudge cells. This difference could be due to sampling error, because hMPV RNA was detected in only a patchy distribution even in the open lung biopsy sample. It is also possible, however, that smudge cells could be reactive in hMPV infection. The present findings are also distinct from those found in experimental hMPV infection of nonhuman primates. In that situation, hMPV replication occurs mainly in ciliated epithelial cells and rarely in type I pneumocytes, and histological assessment shows that erosive and inflammatory changes are confined to the conducting airways [24]. Whether these differences reflect species specificity, disease severity, host immune status, or other factors requires further study. Similarly, although the results of studies of monkeys support a primary role for a new coronavirus in SARS, the present findings again raise the possibility that hMPV contributes to more-severe respiratory tract illness, including SARS [12, 13]. Although the pattern of acute lung injury with smudge cell formation appears distinctive for hMPV infection, at least in the immunocompromised host, additional samples will be needed to more fully determine the diagnostic value of this pattern.

In general, the detection of virus in BAL fluid samples is not sufficient (without concomitant histopathological or clinical presentation) to establish a diagnosis of infection. Nonetheless, this approach has been used to achieve prompt and accurate diagnoses in patients with possible respiratory tract infection. The highest diagnostic yield and impact of BAL fluid sample analysis on therapeutic decisions has been found in immunocompromised hosts, and the combination of BAL with transbronchial biopsy provides an additional diagnostic yield [25]. Under these circumstances, detection of common respiratory tract viruses (influenza virus, parainfluenza virus, RSV, rhinovirus, and ad-

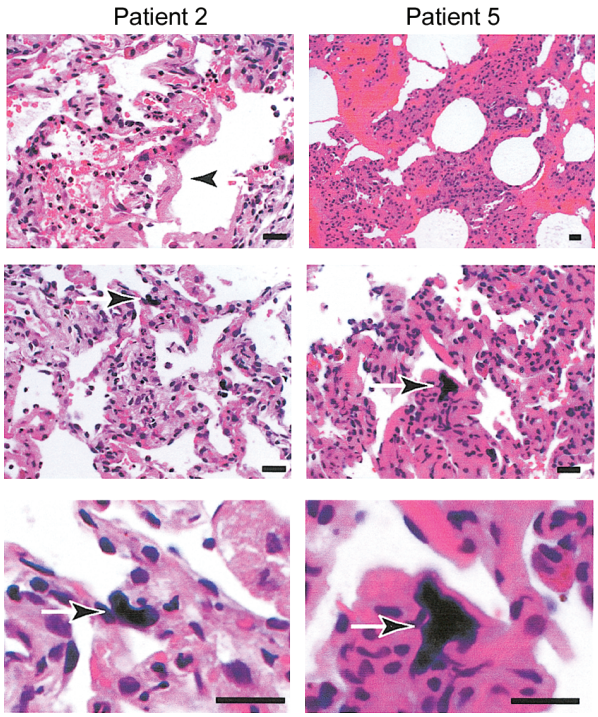

Figure 4. Photomicrographs of human metapneumovirus (hMPV) infection in lung tissue. Representative photomicrographs are shown for hematoxylin-eosin staining of transbronchial biopsy samples from patients with hMPV infection (patients 2 and 5). Arrows indicate the hyaline membrane formation *(first row)* and smudge cells *(second row and corresponding higher magnification in third row)*. Scale bars, 20 μm.

enovirus) is similar to that in the present study (~3% of cases by use of conventional detection methods) [1, 26]. These respiratory tract viruses have been recently recognized as an important cause of lower respiratory tract infection in immunocompromised patients, and their detection may alter the clinical course of the illness [1, 27, 28]. Among these viruses, RSV in particular has been associated with high mortality (26%–100%), especially in bone marrow transplant recipients [27, 29, 30]. In our study population, which was weighted toward immunocompromised patients, hMPV (detected by PCR) was found as frequently as other common respiratory tract viruses (detected by DIFA and culture). Although the relative insensitivity of standard lab tests may underestimate the occurrence of other viruses, this shortcoming should not detract from the importance of using the detection of hMPV to improve patient care. In particular, the detection of hMPV in BAL fluid samples in a proper clinical setting may prevent these high-risk patients from undergoing additional diagnostic procedures and treatments and thereby avoid their attendant morbidity and costs. Whether there is an additional benefit conferred by instituting antiviral treatment still needs to be determined. For RSV, combination treatment with ribavirin and anti-RSV antibody may improve the clinical outcome in immunocompromised patients, but no treatment has yet been tested in vivo for hMPV infection [29, 31, 32]. The results of the present study provide a further rationale for the development of anti-hMPV antibody and other possible therapies to intervene in what appears to be a severe respiratory tract illness. Although we were able to identify only 6 patients with hMPV infection, the consistent clinical features and characteristic pattern of histopathological changes in these patients strongly indicate a clinical significance.

In summary, hMPV is a significant cause of lower respiratory tract infection and is associated with morbidity in the immunocompromised host. We recommend that rapid assessment by PCR for hMPV in BAL fluid samples be included in the evaluation of these patients. A positive result (combined with the absence of other respiratory tract viruses, such as adenovirus) can be correlated with histopathological findings of smudge cells in the background of acute and organizing lung injury to suggest the diagnosis of lower respiratory tract infection due to hMPV.

References

1. Rabella N, Rodriguez P, Labeaga R, et al. Conventional respiratory viruses recovered from immunocompromised patients: clinical considerations. Clin Infect Dis **1999**; 28:1043–8.
2. Raboni SM, Nogueira MB, Tsuchiya LRV, et al. Respiratory tract viral infections in bone marrow transplant patients. Transplantation **2003**; 76:142–6.
3. Garbino J, Gerbase MW, Wunderli W, et al. Respiratory viruses and severe lower respiratory tract complications in hospitalized patients. Chest **2004**; 125:1033–9.
4. Jain P, Sandur S, Meli Y, Arroliga AC, Stoller JK, Metha AC. Role of flexible bronchoscopy in immunocompromised patients with lung infiltrates. Chest **2004**; 125:712–22.
5. Hohenadel IA, Kiworr M, Genitsariotis R, Zeidler D, Lorenz J. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broad spectrum antibiotic and antifungal regimen. Thorax **2001**; 56:115–20.
6. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med **2001**; 7:719–24.
7. Jartti T, van den Hoogen BG, Garofalo RP, Osterhaus ADME, Ruuskanen O. Metapneumovirus and acute wheezing in children. Lancet **2002**; 360:1393–4.
8. Boivin G, Abed Y, Pelletier G, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J Infect Dis **2002**; 186:1330–4.
9. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. J Infect Dis **2003**; 187: 785–90.
10. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med **2004**; 350:443–50.
11. van den Hoogen BG, van Doornum GJJ, Fockens JC, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. J Infect Dis **2003**; 188:1571–7.
12. Chan PKS, Tam JS, Lam C-W, et al. Human metapneumovirus detection in patients with severe acute respiratory syndrome. Emerg Infect Dis **2003**; 9:1058–63.
13. Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. N Engl J Med **2003**; 348:1995–2005.
14. Agapov E, Sumino K, Gaudreault-Keener M, Storch GA, Holtzman MJ. Diversity of human metapneumovirus infection in a US population (abstract W7-6). In: Program and abstracts of the American Society for Virology 23rd annual meeting (Montreal, 2004). Milwaukee, WI: American Society for Virology, **2004**:84.
15. Storch GA. Respiratory infections. In: Storch GA, ed. Essentials of diagnostic virology. New York: Churchill Livingstone, **2000**:59–78.
16. Veness-Meehan KA, Pierce RA, Moats-Staats BM, Stiles AD. Retinoic acid attenuates O_2-induced inhibition of lung septation. Am J Physiol Lung Cell Mol Physiol **2002**; 283:L971–80.
17. Walter MJ, Morton JD, Kajiwara N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. J Clin Invest **2002**; 110:165–75.
18. Neilson KA, Yunis EJ. Demonstration of respiratory syncytial virus in an autopsy series. Pediatr Pathol **1990**; 10:491–502.
19. Fuller CE. Fatal pneumonia in an allogeneic bone marrow transplant recipient. Arch Pathol Lab Med **2000**; 124:1557–8.
20. Hasleton PS. Viral infections. In: Hasleton PS, ed. Spencer's pathology of the lung. 5th ed. New York: McGraw-Hill, **1996**:139–78.
21. Ohori NP, Michaels MG, Jaffe R, Williams P, Yousem SA. Adenovirus pneumonia in lung transplant recipients. Hum Pathol **1995**; 26:1073–9.
22. Ladenheim HS, Mistchenko AS, Drut R. Expression of early and late adenoviral proteins in fatal adenovirus bronchopneumonia. Pediatr Pathol Lab Med **1995**; 15:291–8.
23. Pinto A, Beck R, Jadavji T. Fatal neonatal pneumonia caused by adenovirus type 35. Arch Pathol Lab Med **1992**; 116:95–9.
24. Kuiken T, Van den Hoogen BG, van Riel D, et al. Experimental human metapneumovirus infection of cynomolgus macaques *(Macaca fascicularis)* results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. Am J Pathol **2004**; 164:1893–900.
25. Rano A, Augusti C, Jimenez P, et al. Pulmonary infiltrates in non-HIV immunocompromised patients: a diagnostic approach using non-invasive and bronchoscopic procedures. Thorax **2001**; 56:379–87.
26. Connolly MG, Baughman RP, Dohn MN, Linnemann CCJ. Recovery of viruses other than cytomegalovirus from bronchoalveolar lavage fluid. Chest **1994**; 105:1775–81.

27. Wendt CH, Fox JMK, Hertz MI. Paramyxovirus infection in lung transplant recipients. J Heart Lung Transplant **1995**; 14:479–85.
28. Billings JL, Hertz MI, Wendt CH. Community respiratory virus infections following lung transplantation. Transpl Infect Dis **2001**; 3:138–48.
29. Whimbey E, Champlin RE, Englund JA, et al. Combination therapy with aerosolized ribavirin and intravenous immunoglobulin for respiratory syncytial virus disease in adult bone marrow transplant recipients. Bone Marrow Transplant **1995**; 16:393–9.
30. Krinzman S, Basgoz N, Kradin R, et al. Respiratory syncytial virus-associated infections of adult recipients of solid organ transplants. J Heart Lung Transplant **1998**; 17:202–10.
31. Ghosh S, Champlin RE, Englund JA, et al. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous immunoglobulin. Bone Marrow Transplant **2000**; 25:751–5.
32. Wyde PR, Chetty SN, Jewell AM, Boivin G, Piedra PA. Comparison of inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. Antivir Res **2003**; 60:51–9.