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Pathology of Human Metapneumovirus Infection: Insights into the Pathogenesis of a Newly Identified Respiratory Virus

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ABSTRACT

Human metapneumovirus (hMPV) is a recently discovered human virus that causes significant respiratory infections. Pathologic features of hMPV infection have not been described. A total of 1257 pediatric respiratory samples submitted for routine clinical virologic testing were additionally tested for hMPV by reverse transcriptase polymerase chain reaction (PCR). Pathology specimens, available in 6 of 53 hMPV-positive patients, were examined by light and electron microscopy and included 6 bronchoalveolar lavage (BAL) and 3 lung biopsy specimens from 6 patients (3 girls and 3 boys) ranging in age from 1 to 16 years. BAL from three patients performed within 4 days of the positive hMPV assay showed epithelial degenerative changes and eosinophilic cytoplasmic inclusions within epithelial cells, multinucleate giant cells, and histiocytes. Inclusions were not seen in three patients with BAL performed ≥ 1 month from the time of their positive assay. Lung biopsy, performed in three patients, all ≥ 1 month from the time of their positive assay, showed chronic airway inflammation and intraalveolar foamy and hemosiderin-laden macrophages; all three patients had an underlying pulmonary/systemic disorder. Our findings delineate the clinicopathologic features in hMPV-infected patients undergoing anatomic sampling, which may provide diagnostic guidance to a practicing pathologist. Further, they contribute toward understanding the pathogenesis of hMPV infection.

bronchiolitis, children, lung, pathology, ultrastructure, viral inclusions

INTRODUCTION

Human metapneumovirus (hMPV) is a recently discovered human virus, first identified in 2001 in young children with respiratory tract disease [1]. It is a member of the *Paramyxoviridae* family, and the most closely related virus that affects humans is respiratory syncytial virus (RSV). Clinical manifestations of hMPV, ranging from mild-to-severe bronchiolitis and occasionally pneumonia, resemble those of RSV [2–4]. At our institution, hMPV was found in 6.2% of patients who were tested for respiratory viruses [5]. Clinical reports have focused mainly on epidemiologic aspects such as viral prevalence in various populations [2–4, 6,7–10]. Herein, we describe the pathologic features of hMPV infection and provide insight into its pathogenesis.

METHODS

Respiratory specimens submitted to the Virology Laboratory, Children's Hospital, Boston, from Oc-

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tober 1, 2000 to August 31, 2002, were retrospectively tested for the presence of hMPV. A total of 1257 samples from 868 patients, age 0 to 18 years, included nasopharyngeal aspirates (62%), bronchoalveolar washings (11%), respiratory aspirates not otherwise specified (8%), tracheal specimens (6%), sputum (4%), nasal discharge (3%), and other respiratory specimens (6%). Samples were tested for hMPV using reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was purified using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). All the specimens were first tested for hMPV using primers that amplify a region of the F-gene [11], using the Access RT-PCR System (Promega, Madison, WI). Optimized conditions were 2 μM $MgSO_4$ with thermal cycling of 45 min at 48°C (reverse transcription), 2 min at 94°C (melting), 40 cycles of PCR for 30 s, 1 min, and 2 min at 94°C (melting), 65°C (annealing), and 68°C (extension), respectively, and 7 min at 68°C (final extension). Specimens that had a product of the expected size in the F-gene PCR were confirmed to contain hMPV with a second PCR using primers specific for the N-gene (5'-ACA CCC TCA TCA TTG CAA CA-3' (forward, bases 106 to 126) and 5'-GCC ATT GTT TTC CTT GCT TC-3'). The RT-PCR conditions were the same as those for the F-gene, except that the annealing temperature was 59°C.

Anatomic pathology records of 53 patients with samples positive for hMPV were examined and all available respiratory specimens from these patients were reviewed. Only pathology specimens received within 4 months before or after a positive hMPV assay were included. Results of concurrent virology laboratory tests were reviewed to exclude coinfection with other viruses. Routine clinical testing was done by direct immunofluorescence (DFA) or by viral culture confirmed by DFA. Additionally, samples from all included patients were retested for RSV using RT-PCR. RNA was purified using the QIAamp Viral RNA Mini Kit (Qiagen). The specimens were then tested for RSV by RT-PCR using primers that amplify a region of the N-gene [11], using the Access RT-PCR System (Promega). Optimized conditions were identical to those used for the hMPV primers, and the annealing temperature was 65°C.

Cytospins of bronchoalveolar lavage (BAL) fluid were made using 1 drop of fluid per slide, and stains included hematoxylin and eosin (H&E) and Wright-Giemsa, as well as iron, oil-red-O, and Grocott methenamine silver (GMS) for a subset of cases. Cellular and background morphology was assessed using H&E and Wright-Giemsa slides. Constituent cells were quantified using 100-cell differential counts. In specimens stained with oil-red-O, lipid index was derived by scoring 100 macrophages as previously described [12].

Tissue specimens were fixed in 10% formalin and paraffin-embedded. Five-micron-thick sections were stained with H&E and examined light microscopically. In two cases, additional tissue was immediately fixed in 2% glutaraldehyde, post-fixed in osmium tetroxide, and embedded in Epon 812. One-micron-thick sections were stained with toluidine blue and examined microscopically to confirm the presence of respiratory epithelium. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with an FEI/Philips EM208S electron microscope (Hillsboro, OR).

Clinical records were reviewed. All aspects of the study were conducted with the approval of our hospital's Institutional Review Board.

RESULTS

Patient details

Respiratory material, six BAL specimens and three lung biopsies, was received for pathologic examination from three boys and three girls from age 1 to 16 years, all sampled within 4 months of their hMPV-positive assay. All six patients had underlying and/or intercurrent diseases. Clinical information (summarized in Table 1) was as follows:

Patient 1

This 14-year-old girl had a history of lung transplantation at age 5 for primary pulmonary hypertension. She was on immunosuppressive therapy and required supplemental oxygen for problems attributed to chronic airway rejection. She underwent a routine surveillance bronchoscopy with BAL; the lavage fluid was hMPV-positive. At the time, the airway mucosa was noted to be friable and pale throughout. No radiographs were obtained.

Patient 2

This 16-year-old girl had a history of obesity, mild mental retardation, and psychosocial problems.

Table 1. Bronchoalveolar lavage findings

	Patient no.					
	1	2	3	4	5	6
Proximity of BAL to hMPV detection	Same day	Same day	4 days after	1 month before	2 months before	2 months after
Age/gender	14 yr/f	16 yr/f	1 yr/f	1 yr/m	3 yr/m	2 yr/m
Underlying disease	S/p bilateral lung transplant for primary pulmonary hypertension	Obesity, psychosocial problems	Complex congenital heart disease and tracheoesophageal fistula s/p repair, scimitar syndrome	Congenital hypothyroidism, chronic lung disease of unknown etiology	Idiopathic pulmonary hemosiderosis	Chronic lung disease due to recurrent pneumonia and reactive airway disease
Viral inclusions?	Yes	Yes	Yes	No	No	No
Ciliacytophthoria?	Yes	Yes	Yes	No	Yes	No
Cell differential	30M,30C,40N	30M,40C,30N	30M,9C,60N,1S	70M,5C,20N,1E,4L	52M,11C,31N,3S,3L	58M,27C,11N,4L
Iron-laden macrophages	Not stained	Numerous	Numerous	Not stained	Numerous	Occasional
Lipid index	89	Not assessed	84	Not assessed	Not assessed	43
Other		Concurrent sample positive for RSV by PCR; Candida in BAL				
Proximity of biopsy to hMPV detection	3 months after				4 months before	1 month after

BAL, bronchoalveolar lavage; C, ciliated epithelial cells; E, eosinophils; f, female; hMPV, human metapneumovirus; L, lymphocytes; M, macrophages; m, male; N, neutrophils; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; s/p, status post; S, squamous epithelial cells; yr, year.

She was admitted with presumed septic shock after a 1-day history of mild headache and one episode of emesis. Initially, blood pressure was 72/48 mmHg; heartrate was 157 beats/min; respiratory rate was 34/min; temperature was 39.7°C; oxygen saturation was 95%. The patient quickly became hypoxic and required intubation. White blood cell count was 2.5 K/μL. The chest radiograph initially showed opacification of the left lower and middle lung fields, then progressed to a total left lung white-out with patchy infiltrates on the right, clinically consistent with acute respiratory distress syndrome. She was treated with supportive care, including vasopressors, and empiric antibiotics. Bronchoscopy and BAL performed on the 11th hospital day, showed erythema of the left mainstem bronchus. Lavage fluid showed *Candida* species and was later determined to contain hMPV. The patient recovered and was discharged after 4 wk of hospitalization.

Patient 3

This 13-month-old girl had a history of complex congenital heart disease with multiple surgical repairs, tracheo-esophageal fistula repair (with suspected gastroesophageal reflux and jejunostomy tube feedings), scimitar syndrome, hypothyroidism, hemivertebrae, and a baseline oxygen requirement of 1/4 L/min. At age 9 months, she was admitted for presumed aspiration pneumonia. At age 12 months, she was admitted overnight for an increasing oxygen requirement, preceded by a 2–3-wk history of increasing nasal congestion and mild respiratory distress. Respiratory rate was 34/min; pulse was 148/min; temperature was 35.3°C. A chest radiograph showed no evidence of pneumonia. Two weeks later, she was readmitted for similar symptoms. White blood count was 8 K/μL. Again, a chest radiograph showed no evidence of pneumonia. The presumptive diagnosis was viral respiratory illness complicating chronic lung disease. A specimen collected on the 3rd hospital day was subsequently determined to be positive for hMPV. Bronchoscopy, performed on the 7th hospital day, revealed a type I laryngeal cleft and an intact tracheo-esophageal pouch. Erythematous airway mucosa was also noted. Lavage fluid from this procedure eventually grew *Pseudomonas* species and rare *Staphylococcus aureus*. The laryngeal cleft was surgically repaired. The patient improved and was able to discontinue oxygen supplementation and breath comfortably, and she was discharged after 1 month. However, 4 days after discharge, she was seen again in clinic for cough, wheezing, and an oxygen requirement of 1/8 to 1/4 L/min, attributed this time to irritation from an inhaled antibiotic preparation.

Patient 4

This 1 2/12-year-old boy with congenital hypothyroidism was born at term, yet was hospitalized in the neonatal intensive care unit with a 3-wk oxygen requirement. At 12 months of age, he had a 2–3 day episode of wheezing that was treated with nebulized albuterol; BAL was performed at this time. He was otherwise well, and presented at 1 2/12 years with a 4–5 day history of cough and decreased playfulness and a 1-day history of worsening respiratory distress. Inspiratory and expiratory wheezes were noted. Respiratory rate was 56/min; temperature was 38.7°C; oxygen saturation was 70–80%; white blood count was 7.8 K/μL. A chest radiograph showed diffuse bilateral perihilar airspace disease involving the right middle lobe, lingula, and upper lobes. Despite a presumptive diagnosis of viral bronchiolitis/pneumonia superimposed upon chronic lung disease, the patient was treated empirically with ceftriaxone and azithromycin. Solumedrol and albuterol were also administered. Specimens collected on hospital days 4 and 12 were positive for hMPV. The patient was discharged after 19 days but was readmitted 5 days later for a recrudescent cough and respiratory distress. After recovery from his acute symptoms, an open lung biopsy was performed.

Patient 5

This 3 3/12-year-old boy was born prematurely at 32 wk. At age 2 4/12 months, he had an episode of severe pulmonary hemorrhage, requiring intubation and transfusion. He was placed on steroids and, although all respiratory symptoms resolved, BAL and serial complete blood counts (CBCs) suggested ongoing hemorrhage. To evaluate the cause, an open lung biopsy was performed at age 2 11/12 and BAL was repeated 2 months later. At age 3 3/12, at the time of the positive hMPV assay, he was admitted for pneumonia after a 1-day history of

lethargy, fever, and cough. White blood cell count was 9.22 K/μL and a chest radiograph showed right perihilar and right upper lobe opacifications. He was treated empirically for community-acquired pneumonia and/or pulmonary hemosiderosis exacerbation with intravenous cefotaxime and oral prednisolone. Of note, no decline in hematocrit occurred, and he was discharged after 4 days.

Patient 6

This 2 1/2-year-old boy had a history of recurrent respiratory tract infections, reactive airway disease (exacerbated by exercise and colds), and recurrent cellulitis. At 5 wk of age, he required 3 wk of intubation for bronchiolitis; at 7 months of age, he required 5 days of intubation for severe respiratory distress; and at age 1 year, he was hospitalized for pneumonia. Evaluation of his immune system, including cellular and humoral immunity, and complement and granulocyte function, was normal. At the time that the hMPV-positive specimen was collected, he was diagnosed with left lower lobe pneumonia which worsened on amoxicillin-clavulinic acid, leading to oxygen saturation of 80–90%. Marked wheezing was noted throughout his 6-day hospitalization. A 14-day course of antibiotics (ampicillin-sulbactam followed by amoxicillin-clavulinic acid) was also administered. Two months later, he was admitted for elective BAL to culture for infectious organisms as part of a workup to rule out aspiration as a cause of recurrent respiratory tract infections. Cultures from the BAL fluid were negative, and results of a pH probe assay and esophagogastroduodenoscopy were within normal limits.

Bronchoalveolar lavage

The BAL specimens were obtained on the same day as the hMPV PCR-positive sample for two patients, 4 days after the positive sample for one patient, and 1 month before, 2 months before, and 2 months after the positive sample in the remaining patients.

The three BAL specimens sampled within 4 days of the date of the hMPV-positive specimen collection all showed numerous granulocytes and macrophages. Differential cell counts are summarized in Table 1. In all three cases, many respiratory epithelial cells showed pyknotic or poorly stained nuclei, hypereosinophilic granular cytoplasm with occasional vacuolization, and loss of cilia (Fig. 1). Degenerating or necrotic respiratory epithelial cell cytoplasm frequently showed an unusual conformation, appearing as elongated strands of granular material which gave the cells the appearance of having a tail. Occasionally, fragments of cytoplasm including ciliacytophthoria (apical fragments of cytoplasm showing cilia) were identified. Cytoplasmic inclusions were seen in respiratory epithelial cells from all three patients. In two samples (patients 1 and 2), epithelial cells showed discrete glassy red round cytoplasmic inclusions measuring 3–4 μm and, in the other sample, there were faint red cytoplasmic structures that were similar but less well defined. In two specimens (patients 1 and 3), similar inclusions were seen in multinucleate giant cells. In all specimens, inclusions were seen in cells that were definitely (patient 2) or probably (patients 1 and 3) macrophages. In all specimens, a subset of respiratory epithelial cells showed reactive changes including nuclear enlargement, open chromatin, plump cytoplasm, and increased cohesion. All specimens also had abundant mucus. Fungal organisms resembling *Candida* species were seen in one specimen (patient 2). Iron-positive macrophages were numerous in the two BALs stained for iron. Lipid index in the two oil-red-O-stained samples was 84 and 89.

In the patient whose BAL was performed 1 month before the hMPV-positive assay (patient 4), the specimen showed degenerating epithelial cells and increased lymphocytes. The patient whose specimen was collected 2 months before the positive assay (patient 5) showed increased neutrophils, ciliacytophthoria, and squamous metaplasia. The patient whose specimen was collected 2 months after the positive assay (patient 6) showed abundant hemosiderin, reactive respiratory epithelial cells, and abundant mucus. The remaining cytologic features are summarized in Table 1.

HMPV-positive laboratory samples from all six patients were negative for concurrent viruses (including RSV, influenza A and B adenovirus, parainfluenza virus (PIV) 1, 2, and 3, and herpes simplex virus types 1 and 2) by routine laboratory assays. One sample (patient 2) was positive for RSV when retested using PCR.

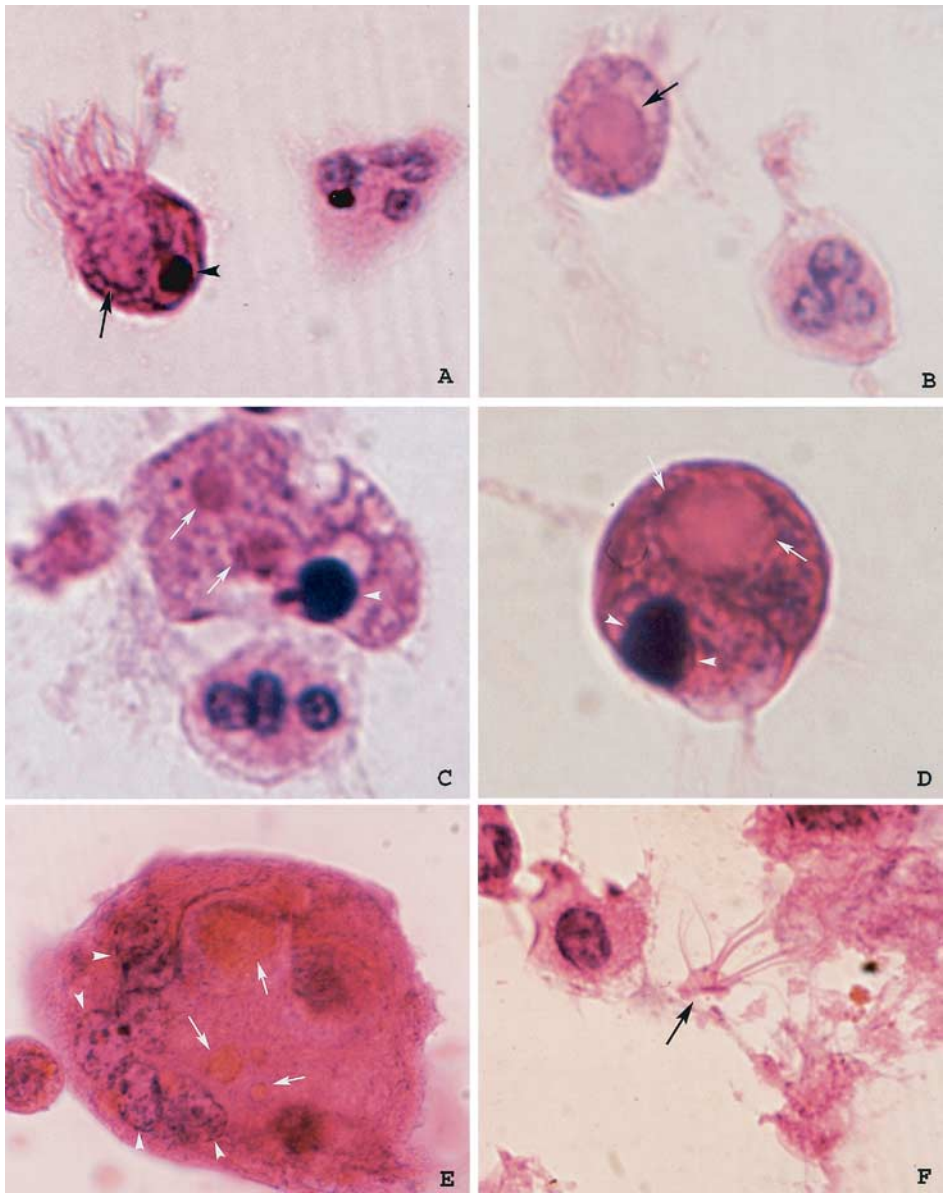

Figure 1. Hematoxylin-and-eosin-stained bronchoalveolar lavage (BAL) specimens, showing **(A)** a glassy red cytoplasmic inclusion (arrow) within a nucleated (arrowhead) ciliated respiratory epithelial cell (patient 1) and **(B)** a red cytoplasmic inclusion (arrow) in a ciliated cell without a visible nucleus (patient 1). Inclusions (arrows) were also seen in **(C,D)** nonciliated mononuclear (arrowheads) cells, representing degenerating epithelial cells or macrophages (patient 1), and in **(E)** multinucleate giant cells (patient 1). Ciliacytophthoria (**F,** arrow) was seen in all acutely infected patients (patient 3). (Original magnification, ×1000.)

Lung tissue: light and electron microscopic examination

Lung biopsy specimens from three patients were examined. Tissue obtained 1 month after the positive assay in one patient (patient 4) showed features of lipoid pneumonia, with numerous intraalveolar foamy macrophages and cholesterol clefts (Fig. 2). There were also expanded bronchus-associated lymphoid tissue and occasional hemosiderin-laden macrophages. Viral inclusions were not seen. Electron microscopic examination showed hyperplastic type 2 pneumocytes, many containing cholesterol crystals and an increase in alveolar septal lymphocytes. No viral particles were observed.

Tissue sampled 4 months before the positive assay in another patient (patient 5, with idiopathic pulmonary hemosiderosis) showed lymphocytic bronchiolitis with areas of follicle formation. Focal intraalveolar fibrin, organizing pneumonitis, and numerous intraalveolar hemosiderin-laden macrophages were also present. Electron microscopic examination in this case showed intraalveolar fibrin, a moderate number of intraepithelial cholesterol crystals, and increased interstitial mast cells. No viral particles were seen.

Tissue sampled 3 months after the positive assay in another patient (patient 1, with a history of lung transplantation) showed acute and chronic

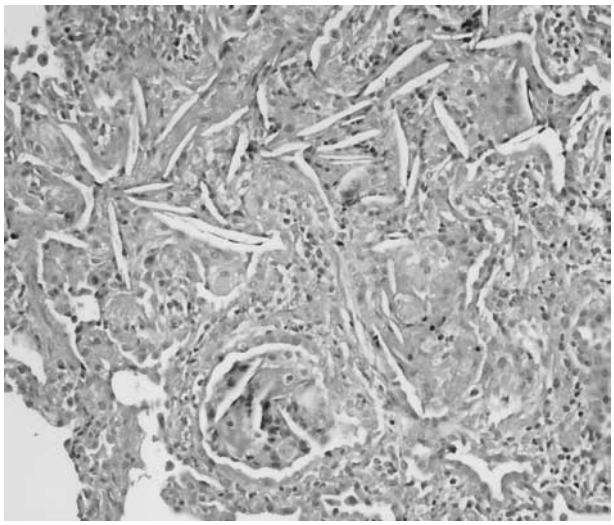

Figure 2. Lung biopsy 1 month after a positive human metapneumovirus (hMPV) assay (patient 4) showed lipoid pneumonia characterized by collections of intraalveolar foamy macrophages with cholesterol clefts. (Original magnification, ×200.)

bronchitis/bronchiolitis and chronic bronchitis with focal squamous metaplasia by light microscopic examination.

DISCUSSION

This study describes the pathologic features of hMPV respiratory tract infection. Findings on BAL include respiratory epithelial cell degeneration and/or necrosis with ciliacytophthoria and round red cytoplasmic inclusions, in a background of hemosiderin-laden macrophages, abundant neutrophils, and prominent mucus. Common to lung biopsy specimens sampled after recent hMPV infection were chronic inflammatory changes of the airways and intraalveolar foamy and hemosiderin-laden macrophages. All samples studied were from patients with preexisting or superimposed lung disease.

This study represents the first anatomic description of hMPV infection. The cytologic and histologic features identified support the clinical impression that hMPV primarily affects airway epithelium. The findings suggest a pathogenetic mechanism that includes direct infection of airway epithelial cells resulting in degeneration, necrosis, neutrophilic response, and increased mucus production. Tissue destruction is sufficiently severe to result in local hemorrhage. Later stages of disease appear to include expansion of peribronchiolar lymphoid tissue, squamous metaplasia, hemosiderin, and accumulation of intraalveolar foamy macrophages, indicating chronic/healing airway inflammation with a degree of concomitant airway obstruction and impairment of the mucociliary escalator. The features correlate well with the bronchiolitis and wheezing noted clinically in patients with hMPV [3, 4, 13]. Further, the findings suggest that at least some of the "pneumonia" observed clinically in hMPV-infected patients may represent postobstructive or "lipoid" pneumonia.

Our findings indicate that morphologic assessment of BAL cytology may contribute to the diagnosis of hMPV. The pathology of hMPV infection shares some morphologic overlap with other viruses that may produce red cytoplasmic inclusions within airway epithelial cells. Such inclusions may be seen with infection by RSV, PIV, and measles virus.

RSV is the most commonly isolated respiratory pathogen in children, both at our hospital [5] and others. It may cause bronchiolitis, characterized by airway epithelial necrosis, desquamation, and hyperplasia/metaplasia, or pneumonitis, characterized by patchy necrosis, hyaline membranes, and/or a pattern of giant cell pneumonia. Inclusions are seen only in a subset of cases, most characteristically in the cytoplasm of respiratory epithelial cells [14]. Reports describing the pathologic features of RSV and other viruses are often limited due to a lack of detailed microscopic description and due to a lack of rigorous testing for concurrent viral infection. As with the patients in our study, biopsy material is almost invariably from patients with immune compromise and/or other significant concurrent disease.

PIV has been, prior to the discovery of hMPV, considered the second most common cause of viral infection of the lower respiratory tract in children. Its major manifestations are laryngotracheobronchitis (croup), caused most frequently by type 1 PIV, and pneumonia, caused most frequently by type 3. Histologic features may include airway epithelial denudation, peri-airway lymphocytic inflammation, and/or interstitial pneumonia, sometimes with prominent multinucleated giant cells lining alveoli. Rare cases may show eosinophilic cytoplasmic inclusions in respiratory epithelial cells or giant cells. These are said to be small and

less distinct, for example, than measles inclusions [14–16]. Measles virus infection, although important historically and in unvaccinated populations, is not often seen in the United States. Mild subclinical pneumonia is the most common respiratory component of measles infection, and severe pneumonia occurs rarely. A pattern of giant cell pneumonia is the most commonly described histologic feature. Giant cells are often numerous and characteristically contain many (10–60) nuclei. Viral inclusions are frequently difficult to identify and when seen are present in the cytoplasm of giant cells, airway epithelial cells, and/or mucosal gland epithelium [14].

Another infection that enters the differential diagnosis of hMPV is upper respiratory tract ameboflagellates, which have been described in nasal aspirates and sputum, particularly in allergy and asthma patients. Flagella from these organisms may be mistaken for cilia. Ameboflagellates may also show reddish cytoplasmic inclusion bodies, nuclear pyknosis and karyorrhexis, and cytoplasmic disintegration [17,18]. The degenerating cells seen in the hMPV-positive patients from this series can be distinguished from ameboflagellates by their straight cilia of uniform length.

The method of case ascertainment is a strength of this study. It did not ensure that all anatomic pathology specimens were sampled on the exact same day as specimens collected for laboratory viral testing. However, this ascertainment method yielded cases without bias toward certain pathologic features, as, for example, would have occurred had only anatomically suspected cases of viral infection been tested for hMPV. It also allowed for the examination of samples from different timepoints in the course of hMPV infection, including a healing phase when viral assay may or may not be positive.

The study design further permitted us to conclude that anatomic specimens from patients harboring hMPV come from a wide age range among pediatric patients, without an apparent gender predilection. They also appear to be associated with a high rate (100% at this tertiary care hospital) of a major preexisting or coexisting disease, suggesting that hMPV infection alone may not be enough to prompt BAL or biopsy. The coexisting disease was likely responsible for some of the pathologic changes seen in this series. However, despite the disparate nature of the patients' underlying diseases, many common features were seen among their specimens, suggesting a causative role for hMPV. That cytoplasmic inclusions and features of airway-centered disease might be expected in this RSV-related virus further support the validity of the findings.

To summarize, this study demonstrates the anatomic pathologic features in hMPV infection. These findings are important because they may provide diagnostic information to a practicing pathologist. Further, they contribute toward understanding the pathogenesis of this recently discovered respiratory virus.

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REFERENCES

1. 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;7:719–724.
2. 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; 186:1330–1334.
3. Freymouth F, Vabret A, Legrand L, et al. Presence of the new human metapneumovirus in French children with bronchiolitis. Pediatr Infect Dis J 2003;22:92–94.
4. Stockton J, Stephenson I, Fleming D, Zambon M. Human metapneumovirus as a cause of community-acquired respiratory illness. Emerg Infect Dis 2002;8:897–901.
5. McAdam, AJ, Hasenbein, ME, Feldman, HA (2004) The role of human metapneumovirus in respiratory infections in children. J Infect Dis 190: 20–26
6. Peris JS, Tang WH, Chan KH, Khong PL, Guan Y, Lau YL, Chiu SS. Children with respiratory disease associated with metapneumovirus in Hong Kong. Emerg Infect Dis 2003;9: 628–633.
7. Esper F, Boucher D, Weibel C, Martinello RA, Kahn JS. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics 2003;111(6 Pt 1):1407–1410.
8. Viazov S, Ratjen F, Scheidhauer R, Fiedler M, Roggendorf M. High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J Clin Microbiol 2003;41:3043–3045.
9. Peret TC, Boivin G, Li Y, et al. Characterization of human metapneumoviruses isolated from patients in North America. J Infect Dis 2002;185:1660–1663.
10. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human

metapneumovirus infections in young and elderly adults. J Infect Dis 2003;187:785–790.
11. Osiowy C. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription-PCR assay. J Clin Microbiol 1998;36:3149–3154.
12. Corwin RW, Irwin RS. The lipid-laden alveolar macrophage as a marker of aspiration in parenchymal lung disease. Am Rev Respir Dis 1985;132:576–581.
13. Jartti T, van den Hoogen B, Garofalo RP, Osterhaus AD, Ruuskanen O. Metapneumovirus and acute wheezing in children. Lancet 2002;360:1393–1394.
14. Zuppan CW, Robinson CC, Langston C 1995 Viral pneumonia in infants and children FB Askin C Langston HS Rosenberg J Bernstein Pulmonary Disease. Perspectives in Pediatric Pathology, Vol. 18 Karger Basel111–153.
15. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 31-1996. An 18-month old immunosuppressed boy with bilateral pulmonary infiltrates. N Engl J Med 1996;335:1133–1140.
16. Wendt CH, Weisdorf DJ, Jordan MC, Balfour HH Jr, Hertz MI. Parainfluenza virus respiratory infection after bone marrow transplantation. N Engl J Med 1992;326:921–926.
17. Bryan WTK, Bryan MP. Structural changes in the ciliated epithelial cells during the common cold. Trans Am Acad Ophthalmol Otolaryngol 1953;3:297–301.
18. Ribas A, Jou C, Escobar J. Ciliocythophthoria and ameboflagellates. Acta Cytol 2003;47:705–708.