Human Metapneumovirus and Community-Acquired Pneumonia in Children

Pen-Yi Lin, MD; Tzou-Yien Lin, MD; Yhu-Chering Huang, MD, PhD; Kuo-Chien Tsao[1], MSc; Ya-Ling Huang[1], MSc

Background: Human metapneumovirus (hMPV) was first recognized in the Netherlands in 2001 from nasopharyngeal aspirate samples and was associated with respiratory tract illness in the pediatric population. This was the first report of metapneumovirus infections in community-acquired pneumonia in Taiwan.

Methods: A total of 116 nasopharyngeal aspirate samples from patients with community-acquired pneumonia was examined by reverse transcriptase polymerase chain reaction (RT-PCR). Other respiratory tract pathogens were also examined. The clinical characteristics and laboratory data were analyzed.

Results: Out of the 116 patients, potential causative agents were detected in 95 (81.9%) patients. A total of six human metapneumovirus RT-PCR positive samples was identified. All of these had evidence of coinfection with bacteria (3 *Streptococcus pneumoniae,* 2 *Mycoplasma pneumoniae,* 1 *Chlamydia pneumoniae*). Coinfection with other respiratory viruses was also observed in two cases (1 influenza A, 1 parainfluenza type 3). The age distribution was seven to 11 years except for one patient who was two years of age (Case 1). The most common clinical findings were fever (6/6, 100%), cough (6/6,100%), rhinorrhea (5/6, 83.3%), rales (5/6, 83.3%) and wheezing (1/6, 16.7%). Chest radiographs revealed four with lobar patches and two with interstitial infiltrations. The mean duration of hospital stay was 5.5 ± 2.8 days. All patients made a complete recovery.

Conclusions: hMPV was identified in 5.2% of patients with community-acquired pneumonia. Our data showed a high rate of coinfection with hMPV in community-acquired pneumonia. Human metapneumovirus infection, like other respiratory viruses, may predispose to secondary bacterial pneumonia.

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Key words: human metapneumovirus, community-acquired pneumonia.

Community-acquired pneumonia is a major cause of morbidity and mortality in children. Several bacteria, viruses and combinations of both can cause the disease. Mixed viral-bacterial infections as well as viral-viral infections and dual bacterial infections have been described.[1,2] In 2001, van den Hoogen et al.[3] reported the isolation of a paramyxovirus, human metapneumovirus (hMPV), in children with respiratory tract disease. Early reports indicated that hMPV was found in approximately 10% of children;

From the Division of Infectious Disease, Department of Pediatrics, Chang Gung Children's Hospital, Taipei; [1]Department of Clinical Pathology, Chang Gung Memorial Hospital, Taipei.
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Correspondence to: Dr. Tzou-Yien Lin, Division of Pediatric Infectious Disease, Department of Pediatrics, Chang Gung Children's Hospital, 5, Fushing Street, Gueishan Shiang, Taoyuan, Taiwan 333, R.O.C. Tel.: 886-3-3281200 ext. 8002; Fax: 886-3-3288957; E-mail: pidlin@cgmh.org.tw

most children have serologic evidence of infection by five years of age, and the emerging picture of clinical symptoms associated with hMPV infection appears to be similar to the disease caused by respiratory syncytial virus (RSV).[4,5] This report describes the characteristics of six children who had community-acquired pneumonia with evidence of hMPV infection in Taiwan for the first time.

METHODS

Between 1 August 2001 and 31 July 2002, the etiology of community-acquired pneumonia was studied in 209 hospitalized children, between 3 months and 18 years of age, at the Department of Pediatrics, Chang Gung Children's Hospital, Taipei. Of these patients, nasopharyngeal specimens from 116 were subsequently tested for hMPV. The diagnosis was based on simultaneous findings of consolidation on chest radiographs and from fever (> 38°C) and/or respiratory symptoms. The radiological diagnosis was made by a pediatric radiologist.

Several bacteriological and virological methods were used to determine the potential cause of the pneumonia. Bacterial infection was identified by blood culture, Gram stain and culture of sputum and/or pleural effusion. For detection of *S. pneumoniae* infection, C-polysaccharide of pneumococcus in urine was measured by NOW™ commercial kit (Binax, Portland, USA). *Mycoplasma pneumoniae* was identified by studying IgM and IgG antibodies in acute and convalescent phase serum samples with ELISA (Savyon, Israel). IgM and IgG antibodies to *Chlamydia pneumoniae* were studied by a microimmunofluorescence method (Virion, Switzerland).

Viral cultures from throat swabs and/or nasopharyngeal aspirates were performed using Hep2, MRC5, MDCK, MK2, and RD cells according to routine procedures. Viral antigens (RSV, influenza A and B virus, parainfluenza virus types 1, 2, 3, and adenovirus) in nasopharyngeal aspirates were detected by immunofluorescent assay. These seven virus specific serum antibody titers from acute and convalescent serum samples were also determined by the complement fixation (CF) method.

hMPV PCR: A 170-bp segment of the L gene spanning nucleotides 11321 to 11490 of the hMPV genome was amplified by using a single-step reverse transcription polymerase chain reaction (RT-PCR) with the modified procedures described previously.[6] Briefly, viral RNA was extracted with the QIAmp Viral RNA kit (Qiagen, Chatsworth, CA, USA) and amplified with the OneStep RT-PCR kit (Qiagen) with antisense primer L7 (5'-CACC-CCAGTCTTTCTTGAAA-3'; positions 11471 to 11490) and sense primer L6 (5'-CATGCCCAC-TATAAAA GGTCAG-3'; positions 11321 to 11342) under the following conditions: reverse transcription at 50°C for 30 min; an initial PCR activation step at 95°C for 10 min; 40 subsequent PCR cycles with melting at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 60 s. The reactions were carried out in a 50-μl format by using a PCR Master Mix containing RT-PCR enzyme mix (Omniscript and Sensiscript reverse transcriptases and HotStartTaq DNA polymerase), each deoxynucleoside triphosphate at a concentration of 400 μM, primers (0.9 μM each) and optimized buffer components. Finally, the amplified product was analyzed by electrophoresis on an agarose gel after ethidium bromide staining, and the sizes of the amplicons were compared with standard molecular size markers. To validate the amplification process and to exclude the presence of carryover contamination, positive and negative controls were run in each PCR. All samples were tested at least in duplicate.

RESULTS

The patients with community-acquired pneumonia included in this study comprised 116 children hospitalized from August 1, 2001 to July 31, 2002. Of these, 55 (47.4%) were males and 61 (52.6%) females, and their ages ranged from 3 months to 18 years with a mean of 4.5 ± 2.5 years (Fig. 1). Potential causative agents were detected in 95 (81.9%) patients. Evidence of viral infection was demonstrated in 45 (38.8%) children including 13 (28.9%) with RSV, 13 (28.9%) with adenovirus, six (13.3%) with hMPV, six (13.3%) with parainfluenza virus type 2, five (11.1%) with influenza A, five (11.1%) with parainfluenza virus type 1, five (11.1%) with parainfluenza virus type 3, and one (2.2%) with influenza B. Eighty four (72.4%) had bacterial infections including 45 (38.8%) with *S pneumoniae,* 44 (37.9%) with *Mycoplasma pneumoniae,* five (4.3%) with *Chlamydia pneumoniae,* and one (0.9%) with *Staphylococcus aureus.* Forty one

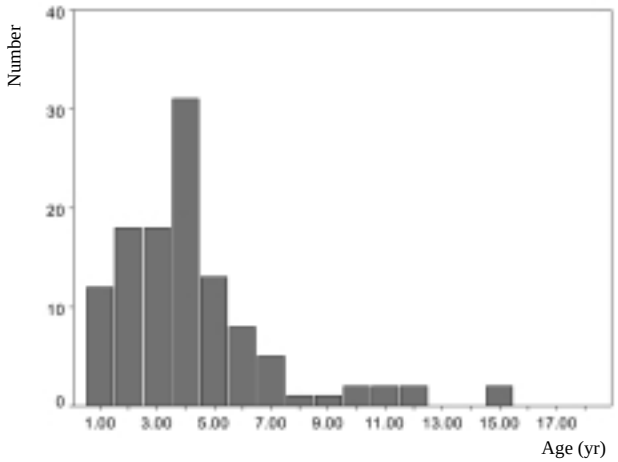

Fig. 1 Age distribution of patients with community-acquired pneumonia.

(35.3%) cases had evidence of mixed viral-viral or viral-bacterial infections (Fig. 2).

A total of six hMPV RT-PCR positive samples was identified. All of these had evidence of coinfection with bacteria (3 *S. pneumoniae*, 2 *Mycoplasma pneumoniae*, 1 *Chlamydia pneumoniae*) (Table 1). The age distribution was seven to 11 years except for one patient who was two years of age (Case 1). Coinfection with other respiratory viruses was also observed in two cases (influenza A and parainfluenza type 3) but no coinfection with RSV was found. Most of the cases (4/6, 66.7%) occurred during winter, from October to January. The most common clinical symptoms were fever (6/6, 100%), cough (6/6,100%), rhinorrhea (5/6, 83.3%), rales (5/6, 83.3%) and wheezing (1/6, 16.7%). Two patients developed mild respiratory distress, which was relieved after being treated with bronchodilators and oxygen. Chest radiographs revealed four with lobar patches and two with interstitial infiltrations. Three children with pneumococcal coinfections had a C reactive protein concentration greater than 100 mg/L and one had a white blood cell count greater than 15 x 10^9/L. In the hospital, five cases were initially treated with macrolide, two patients with additional cefuroxime and one with penicillin alone. No antiviral agents were prescribed for these cases. The mean duration of hospital stay was 5.5 $\pm$ 2.8 days, and there was no significant difference between this and patients with other etiologies (7.2 $\pm$ 4.2 days, p =

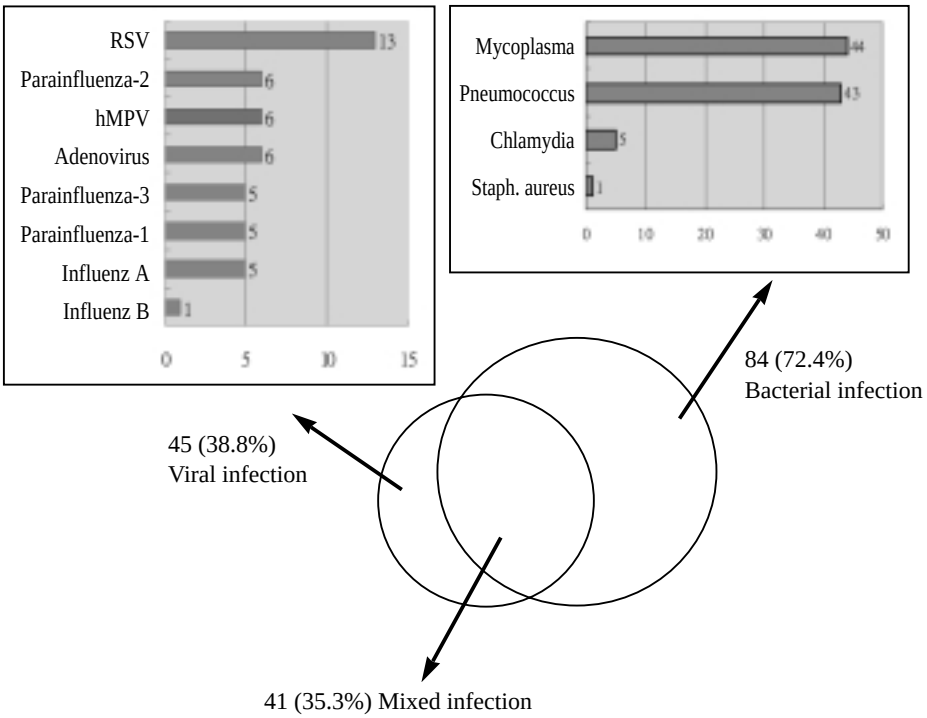

Fig. 2 Etiology of 95 patients with community-acquired pneumonia. A total of six (5.2%) patients were infected with hMPV.

Table 1. Positive Test for hMPV in Children with Coinfection.

Case no.	Gender	Age (yr)	Bacterial Coinfection	Viral Coinfection	Chest Radiography	Hospitalization (Days)
1	M	2	Chlamydia		Interstitial pneumonia	3
2	F	8	Pneumococcus		Lobar pneumonia	8
3	F	8	Mycoplasma		Interstitial pneumonia	4
4	M	9	Mycoplasma		Lobar pneumonia	4
5	F	11	Pneumococcus	Influenza A culture	Lobar pneumonia	4
6	M	7	Pneumococcus	Parainfluenza type 3	Lobar pneumonia	10

Abbreviations: M: male; F: female.

0.249). None of these patients were admitted to an intensive care unit. At the follow up visit, one to two weeks after discharge, all patients had made a complete recovery.

DISCUSSION

This article presents data on the coinfection of hMPV with other respiratory illness pathogens in community-acquired pneumonia. Coinfection of hMPV with other pathogens has been described previously. Fabrizio et al.[6] reported that one-third of hMPV infected children were also infected with other respiratory viruses. Bronchiolitis was the most common diagnosis in children with hMPV infection. Greensill et al.[7] observed a 70% coinfection rate with hMPV and RSV, and 90% coinfection among intubated infants admitted to intensive care units. Semple et al.[8] found that infants with bronchiolitis younger than two years of age and with a coinfection of hMPV and RSV have a 10-fold increase in relative risk of admission to a pediatric intensive care unit. These findings suggest that coinfection with hMPV and RSV is common and may be considered a cause of severe lower respiratory tract disease. However, the role of hMPV in the pathogenesis of infections with other respiratory pathogens is not known.

In contrast, Lazar et al.[9] screened 23 children with severe RSV disease and 23 children with mild RSV disease for hMPV. They did not observe any hMPV coinfection in patients with either mild or severe RSV disease. Vicente et al.[10] collected nasopharyngeal specimens from children younger than three years of age with lower respiratory tract infections, during two consecutive winters, and tested them for several respiratory viruses. The incidence of hMPV infection observed was 4.1% and no cases of coinfection were found.[10] Other studies also support the findings that the frequency of coinfection with hMPV and RSV is rare.[11] The coinfection of hMPV with RSV was also not found in our study but there were two children with other respiratory viruses, one with influenza A and the other with parainfluenza 3.

There has been no report of coinfection of hMPV with bacteria in community-acquired pneumonia. Pneumonia in children may often be caused by multiple microbial agents. The rate of mixed viral-bacterial infection in community-acquired pneumonia was 7-30%.[1,2,12,13] Concurrent or preceding upper respiratory viral infections, such as influenza or mycoplasma, are believed to be risk factors for secondary bacterial disease. In this study, six patients with community-acquired pneumonia had hMPV infection and coinfection with *S. pneumoniae, Mycoplasma pneumoniae,* or *Chlamydia pneumoniae.* This result suggests that hMPV, like other respiratory viruses, may predispose to secondary bacterial infection. Furthermore, community-acquired pneumonia with hMPV does not seem to contribute to a longer hospital stay compared with pneumonia and other etiologies in our study (5.5 vs. 7.2 days, p = 0.249).

Infection with hMPV was more common among children younger than two years of age. In the original report by van den Hoogen et al,[3] 27 out of 28 infected children were younger than five years of age and all were diagnosed with acute mild to severe respiratory tract infections.[3] Williams et al. tested 408 nasal-wash specimens, obtained over a 25 year period from otherwise healthy children, with a mean age of 11.6 months and an age range of 1.5 to 50 months, presenting with acute respiratory tract illness, for hMPV. The clinical diagnosis given to 49 children with hMPV infection was bronchiolitis (59%), croup

(18%), pneumonia (8%) and an exacerbation of asthma (14%).[14] However, symptoms and laboratory data associated with hMPV intection exhibit a spectrum virtually indistinguishable from those with RSV disease.[5] Infection with hMPV is not only restricted to very young children and reinfection with hMPV occurs frequently throughout life.[15] Two additional reports on acute respiratory tract infection in adult outpatients had rates of hMPV infection between 2 to 7%.[16,17] In our study, most hMPV infections were found in patients over five years of age, implying that hMPV may predispose to secondary pneumonia in school-aged children. Previous studies have focused only on children younger than three years of age but elderly patients may not have given an accurate picture of the spectrum on hMPV infection.[18,19] However, our relatively small sample size limited the power of analysis. Further studies will to be needed investigation the role of hMPV infection in community-acquired pneumonia in children.

In this study, although the population-based incidence and prevalence cannot be determined from these data, our findings showed hMPV can be identified in 5.2% of patients with community-acquired pneumonia. Our data also indicated a high rate of coinfection with hMPV in community-acquired pneumonia. Nonetheless, hMPV most likely plays a preceding role in acquired pneumonia in school-aged children.

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人類間質肺炎病毒感染引起之社區性肺炎

林本一 林奏延 黃玉成 曹國倩[1] 黃瓊瑰[1] 黃雅玲[1]

背 景： 人類間質肺炎病毒爲 2001 年新發現之呼吸道感染病毒，該病毒於台灣尚無相關之研究報告，本研究探討該病毒感染於兒童社區性肺炎中之角色和臨床表現之分析。

方 法： 收集 2001 年 8 月至 2002 年 7 月期間在長庚兒童醫院因社區性肺炎而住院之病人，以 Reverse transcriptase polymerase chain reaction (RT-PCR) 方式檢測病人之鼻咽檢體是否有人類間質肺炎之感染。分析其臨床表現，實驗室檢查結果，及和其他社區性肺炎致病原之相互關係。

結 果： 共收集 116 個社區性肺炎病人，年齡分布 3 個月至 18 歲，平均 4.52 歲。共有 95 (81.9%) 個人的致病原被確定。病毒感染有 45 人，細菌性感染有 84 (72.4%) 人。其中人類間質肺炎病毒感染有 6 (5.2%) 人。這 6 人中只有一位爲 2 歲，其於爲 8-11 歲之病童。男女各半。感染期間有 4 位在冬季（十月至次年一月）。他們均可發現混合感染的現象，細菌包括 3 位肺炎雙球菌，2 位黴漿菌肺炎，及 1 位肺炎披衣菌。病毒有 1 位 A 型流感，1 位副流感病毒第三型。胸部 X 光片檢查，4 位爲大葉性肺炎，2 位爲間質性浸潤增加。臨床表現包括發燒及咳嗽 (100%)，流鼻水 (83.3%)，肺部囉音 (83.3%)，哮鳴 (16.7%)。平均住院天數 5.5 ± 2.8 天。這些病人在 1 至 2 星期後的門診追蹤皆全部康復而無其他併發症。

結 論： 人類間質肺炎病毒佔感染社區性肺炎原因的 5.2%。它的感染可以發生在學齡以上幼童而不單是嬰幼兒。混合其他呼吸道感染致病原是很常見的現象，包括細菌和病毒。這些混合感染可能是先感染人類間質肺炎病毒感染，之後造成繼發性感染。其在肺炎病理機轉中所扮演的角色仍不清楚。需進一步的研究。

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關鍵字： 人類間質肺炎病毒，社區性肺炎。

長庚兒童醫院 台北院區 兒童感染科；[1]長庚紀念醫院 台北院區 臨床病理科
受文日期：民國94年4月28日；接受刊載：民國94年8月9日
通訊作者：林奏延醫師，長庚兒童醫院 兒童感染科。桃園縣333龜山鄉復興街5號。Tel.: (03)3281200轉8002; Fax: (03)3288957; E-mail: pidlin@cgmh.org.tw