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CASE REPORT

Respiratory Distress in Human Metapneumovirus Infection with High-inflammatory Cytokinemia

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We encountered two human metapneumovirus (HMPV)-infected children who developed respiratory distress due to bronchopneumonia during hospitalization. There was no evidence of abnormal findings in the gastrointestinal or genitourinary tracts or the nervous or mucocutaneous systems. Their respiratory conditions rapidly improved upon the initiation of steroid therapy. Increases and decreases in serum concentrations of various inflammatory cytokines, including interferon-γ, and interleukin-6/10, were evaluated before and after steroid administration. General practitioners should be aware of the possibility of high-inflammatory cytokinemia when examining and treating children with HMPV infection and respiratory distress due to bronchopneumonia.

1. Introduction

Human metapneumovirus (HMPV), a newly discovered pneumovirus of the *Paramyxoviridae* family, was first isolated from young children with acute respiratory tract illness in the Netherlands.[1] The clinical syndromes induced by HMPV infection in children are similar to those caused by respiratory syncytial virus (RSV) infection, ranging from mild respiratory problems to severe cough, bronchiolitis, and pneumonia.[1,2]

Comprehensive detection of HMPV, RSV, and other respiratory viruses can be performed using a real-time reverse transcription (RT)-polymerase chain reaction (PCR) assay that analyzes clinical nasopharyngeal swab specimens.[3] We encountered two HMPV-infected children who developed respiratory distress due to bronchopneumonia during hospitalization. Their respiratory conditions rapidly improved upon the initiation of steroid therapy. Increases and decreases in the serum concentrations of various inflammatory cytokines were evaluated before and after steroid administration. Here, we report the characteristic immune response that was induced by HMPV infection during respiratory distress along with the clinical course.

2. Case reports

2.1. Patient 1

A previously healthy 3-year-old boy presented with a 3-day history of high fever and productive cough. He was referred to the pediatrics department because of prolonged symptoms. The patient was hospitalized on the fourth day after onset. Physical examination on admission revealed a fever (38.4°C) and bilateral coarse inspiratory rales upon auscultation. Respiratory distress was not evident from the respiratory rate (RR; 30 breaths/minute) or percutaneously measured oxygen saturation (SpO_2; 97%) when breathing room air. A chest roentgenogram performed on admission indicated increased linear shadows in both hila without hyperaeration. Laboratory findings included a reduced white blood cell count (WBC; 3200 cells/μL) and a slightly elevated serum concentrations of C-reactive protein (CRP; 1.3 mg/dL; normal range: <0.3 mg/dL), aspartate aminotransferase (AST; 68 IU/L), and lactate dehydrogenase (LDH; 361 IU/L). To determine the etiologic agent, a nasopharyngeal swab sample was collected after the patient's written

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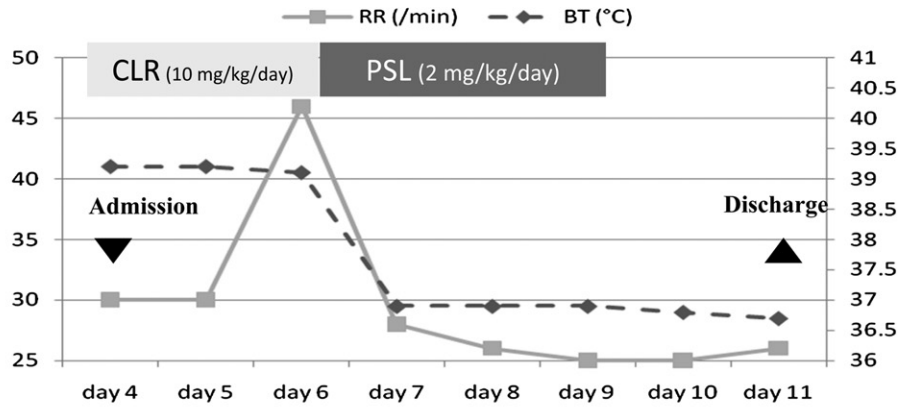
informed consent was signed by his respective guardian/parent. This specimen was sent to the Laboratory of Molecular Epidemiology for Infectious Agents at Kitasato Institute for Life Sciences, and a comprehensive rapid analyses using real-time RT-PCR was performed to detect RSV subgroups A and B, influenza virus types A and B, pandemic (H1N1) 2009 virus, parainfluenza virus types 1–3, rhinovirus, enteroviruses, HMPV, human bocavirus, and adenovirus,[4,5] as well as real-time PCR to identify *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydophila pneumoniae*, and *S pyogenes*.[6,7] The specific primers used to detect HMPV in the real-time RT-PCR analysis included a sense primer (5'-AACCGTGTACTAAGTGA TGCACTC-3') and a reverse primer (5'-CATTGTTTGACCGGCCCCATA A-3'); a specific molecular beacon probe, 6-carboxyfluorescein-GCTGCCCTTTGCCATACTCAATGAACAAACGGCAGC- black hole quencher 1 (213 bp), was also used. The nucleocapsid gene of HMPV (GenBank accession number: AF371337) was used as the RT-PCR target. This test detected HMPV cDNA, but no other respiratory bacterial or viral DNAs were present. Bacterial culturing of the same specimen yielded no causative pathogens.

The patient's vital signs, treatments, and laboratory data during the clinical course are shown in Figure 1A. The patient received oral clarithromycin (10 mg/kg/day) to prevent a secondary bacterial infection, according to the 2007 guidelines adopted for the

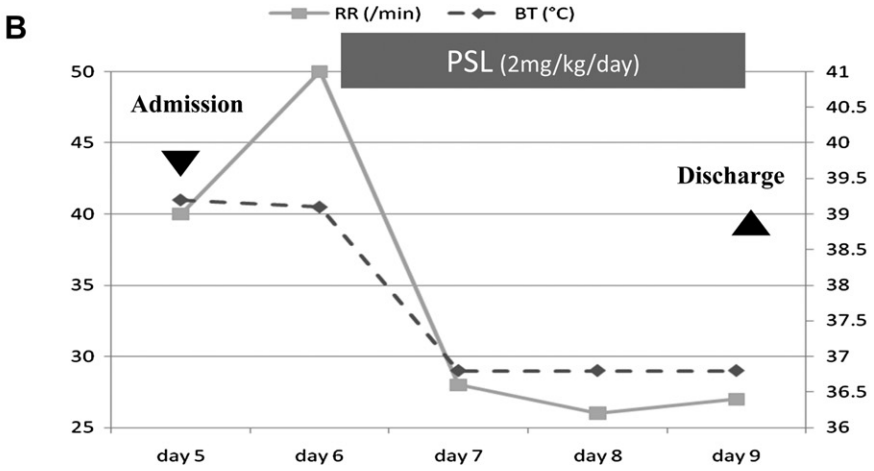
A



SpO_2 (%)	97	97	93	99	98	98	98	98

	day 4	day 6	day 8	day 19
IL-2 (pg/mL)		4.1		0
IL-4 (pg/mL)		5.5		5.9
IL-6 (pg/mL)		13.6		0
IL-10 (pg/mL)		49.1		6.2
TNF-alpha (pg/mL)		4.3		0
IFN-gamma (pg/mL)		27.0		0
IL-17A (pg/mL)		0		20.0
WBC (cells/μL)	3200	4200	13,300	5600
Neutrophils (%)	47.5	40.1	75.2	46.5
Lymphocytes (%)	44.5	46.3	18.1	47.0
CRP (mg/dL)	1.3	0.8	<0.1	<0.1
AST (IU/L)	68	79	50	45
LDH (IU/L)	361	594	411	297
Ferritin (ng/mL)		276	137	27
sIL-2R (U/mL)		931	671	863

Figure 1 Clinical courses of patient 1 (Figure 1A) and patient 2 (Figure 1B), including vital signs, treatments, and laboratory data. Intravenous administration of prednisolone (2 mg/kg/day) for 3 days was initiated on the sixth day after onset. Elevations in serum IFN-γ, IL-6, and IL-10 concentrations resolved after the initiation of steroid therapy. A high serum concentration of IL-17A was demonstrated during the convalescent phase. RR, respiratory rate; BT, body temperature; CLR, clarithromycin; PSL, prednisolone; SpO_2, percutaneously measured oxygen saturation; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; WBC, white blood cells; CRP, C-reactive protein; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; sIL-2R, soluble interleukin-2 receptor.



SpO_2 (%)	95	90	98	98	98
	day 5	day 6	day 9		day 13
IL-2 (pg/mL)		8.3			0
IL-4 (pg/mL)		0			4.9
IL-6 (pg/mL)		14.3			0
IL-10 (pg/mL)		22.8			4.9
TNF-alpha (pg/mL)		7.1			6.3
IFN-gamma (pg/mL)		29.4			6.9
IL-17A (pg/mL)		13.7			17.5
WBC (cells/μL)	4500	4600	8600		9700
Neutrophils (%)		42.5	75.2		39.4
Lymphocytes (%)	27.9	51.0	18.1		48.7
CRP (mg/dL)	1.4	3.4	0.3		<0.1
AST (IU/L)	40	79	49		28
LDH (IU/L)	417	542	384		293
Ferritin (ng/mL)		152	53		27
sIL-2R (U/mL)		1120	718		651

Figure 1 (*continued*).

management of children with respiratory infectious diseases in Japan.[8] On the sixth day after onset, respiratory distress due to bronchopneumonia became evident with tachypnea (RR: 45 breaths/minute), supraclavicular and intercostal inspiratory retraction, and decreased SpO_2 (93%) when breathing room air. Vesicular sounds were also insufficient over both hemithoraces. There was no evidence of abnormal findings in the gastrointestinal or genitourinary tracts or the nervous or mucocutaneous systems. The patient required oxygen supplementation (2 L/min). Testing of the immediately obtained blood specimen indicated increases in AST, LDH, ferritin, and soluble interleukin-2 receptor (sIL-2R) levels. There were no data indicative of bacterial coinfection because the WBC count and CRP level were not significantly elevated. Based on these findings, HMPV infection appeared to induce respiratory distress by eliciting an excessive immune response. Intravenous administration of prednisolone (PSL; 2 mg/kg/day) for 3 days was initiated to inhibit the excessive immune response. The patient's respiratory condition rapidly improved after steroid therapy was initiated without any steroid-related adverse events. On the 11th day, the boy was discharged. No recurrence of respiratory distress was evident at the follow-up assessment performed on the 19th day after onset.

2.2. Patient 2

A previously healthy 4-year-old boy presented to our department with a 4-day history of high fever and productive cough. He was hospitalized on the fifth day after onset. Coarse inspiratory rales were bilaterally audible, but respiratory distress was not evident. A chest roentgenogram performed on admission indicated increased lin'ear shadows in both hila without hyperaeration. A nasopharyngeal swab sample was obtained and sent to the same laboratory as patient 1. The RT-PCR assay detected HMPV cDNA, but no other respiratory viral DNAs were detected. Two bacterial DNAs (*S pneumoniae* and *H influenzae*) were positive, and culturing of the same specimen resulted in the growth of the same organisms. However, these bacterial contributions were considered to be insignificant because WBC and CRP levels were not elevated during hospitalization.

The clinical course and related information are shown in Figure 1B. On the sixth day after onset, respiratory distress due to bronchopneumonia became evident with tachypnea (RR: 52 breaths/minute), supraclavicular and intercostal inspiratory retraction, and decreased SpO_2 (90%) when breathing room air. Vesicular sounds were also insufficient over both hemithoraces. The patient required oxygen supplementation (3 L/minute). Immediate blood testing revealed elevation in his AST, LDH, ferritin, and sIL-2R levels. These findings suggested that the respiratory distress might be induced by an excessive immune response caused by HMPV infection. Intravenous administration of PSL (2 mg/kg/day) for 3 days was initiated to inhibit the excessive immune response. The patient's respiratory condition rapidly improved after steroid therapy was initiated without any steroid-related adverse events. On the 9th day, the boy was discharged, and no recurrence of the distress was evident at a follow-up assessment performed on the 13th day after onset.

2.3. Analysis of the serum cytokine profiles

In these two cases, serum samples were obtained during the acute phase and convalescent phase after the patients' written informed consent was signed by his respective guardian/parent. Circulating levels of seven inflammatory cytokines were measured using the BD CBA kit (Becton-Dickinson Biosciences, San Diego, CA, USA) and the BDTM cytometric bead array system (Becton-Dickinson).[9] We confirmed increases and decreases in the serum concentrations of various inflammatory cytokines, including interferon (IFN)-γ, IL-6, and IL-10, both before and after steroid administration. There were different ratios of IFN-γ to IL-4 in the sera, which were determined to assess T helper (Th) balance in the immune responses (i.e., Th1/Th2) of these two cases. Th2 polarization (IFN-γ/IL-4: 27.0/5.5) was observed in patient 1, while there was a potential Th1 shift (IFN-γ/IL-4: 29.4/0) in patient 2.

3. Discussion

The high serum concentrations of ferritin and sIL-2R, as well as the elevated AST and LDH levels, suggested that excessive immune responses contributed to our patients' respiratory disturbances. Therefore, we determined the circulating concentrations of various inflammatory cytokines (IFN-γ, IL-6, IL-10, etc.) in the acute phase, although we were unable to investigate the cytokine values in the respiratory secretions. A previous retrospective study on circulating cytokines in five HMPV-infected infants reported increases in serum IFN-γ and IL-6 levels.[10] The concentration of the proinflammatory IL-8 cytokine has also been reported as elevated in nasal secretions obtained from 10 HMPV-infected children who presented with the sudden onset of wheezing.[11] In a mouse model, IL-6 and IL-10 levels in bronchoalveolar lavage fluid were significantly higher in HMPV-infected mice than in RSV-infected mice.[12] Accordingly, our findings regarding inflammatory cytokine concentrations are compatible with previous investigations on HMPV infection-induced immune responses.

Thecytokine profiles of the nasal secretions were prospectively determined during primary infection with HMPV ($n = 22$), RSV ($n = 46$), and influenza virus ($n = 19$) in infantswho were brought to emergency departments or outpatient clinics.[13] Influenza induced higher concentrations of IFN-γ, IL-4, and IL-2 than the other infections.[13] Interestingly, infants infected with HMPV demonstrated a lower IFN-γ/IL-4 ratio (approximately half the ratio reported for RSV infection or influenza), suggesting greater Th2 tendencies than infants with other viruses.[13] These observations are also in agreement with the Th2 tendencies reported in a murine model of HMPV or a model infected with an inactivated form of the virus,[14–16] suggesting that HMPV plays a role in exacerbating asthma.[15] Respiratory infections with HMPV or RSV have recently been reported to induce different host responses in mice.[17] Further analysis of the serum cytokine profiles will need to be performed on HMPV-infected children with respiratory failure.

Steroid challenges have been administered to children with RSV infection in several clinical situations. However, the effectiveness of steroid therapy has not been documented in clinical studies.[18,19] In the acute phase of RSV infection in infants, an increase in the plasma cortisol level coincided with decreases in IL-12 and IFN-γ production.[20] The increase in plasma cortisol was also greater in infants with more severe illness and associated with lower levels of IL-12 and IFN-γ production.[20] In another report,[21] the systemic administration of dexamethasone did not have a consistent effect on the concentrations of proinflammatory cytokines in tracheal aspirates from children with severe RSV disease. Inhaled steroids did not demonstrate significant effects to ciliary beat frequency or the ultrastructure of the ciliated respiratory epithelium of infants following viral bronchiolitis.[22] No specific guidelines exist for the treatment of children with HMPV infection caused by respiratory distress. Definitive proof of both the efficacy and safety of systemic steroid administration in HMPV-infected infants being treated for respiratory distress requires a randomized trial.

The interaction between IL-6 and CRP is well documented in cardiovascular diseases.[23] Whereas increases in serum IL-6 concentrations have been observed in the acute phase before the initiation of PSL therapy, markedly elevated CRP levels in the sera were not confirmed at the same time. Therefore, a relationship between IL-6 and CRP was less likely to have occurred in the two cases of respiratory stress and high-inflammatory cytokinemia.

In conclusion, general practitioners should be aware of the possibility of high-inflammatory cytokinemia when examining and treating children with HMPV infection and respiratory distress due to bronchopneumonia.

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References

1. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719–24.
2. Kroll JL, Weinberg A. Human metapneumovirus. *Semin Respir Crit Care Med* 2011;**32**:447–53.

3. Hamano-Hasegawa K, Morozumi M, Nakayama E, Chiba N, Murayama SY, Takayanagi R, Iwata S, et al. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *J Infect Chemother* 2008;**14**:424–32.
4. Takahashi T, Morozumi M, Chiba N, Asami R, Kishii K, Murayama SY, Ubukata K. Co-infection with respiratory syncytial virus subgroup A and *Streptococcus pneumoniae* detected by a comprehensive real-time polymerase chain reaction assay in an elderly patient with community-acquired pneumonia. *J Am Geriatr Soc* 2009;**57**:1711–3.
5. Hasegawa M, Inamo Y, Fuchigami T, Hashimoto K, Morozumi M, Ubukata K, Watanabe H, et al. Bronchial casts and pandemic (H1N1) 2009 virus infection. *Emerg Infect Dis* 2010;**16**:344–6.
6. Morozumi M, Nakayama E, Iwata S, Aoki Y, Hasegawa K, Kobayashi R, Chiba N, et al. Simultaneous detection of pathogens in clinical samples from patients with community-acquired pneumonia by real-time PCR with pathogen-specific molecular beacon probes. *J Clin Microbiol* 2006;**44**:1440–6.
7. Takahashi T, Morozumi M, Okada T, Chiba N, Asami R, Murayama SY, Ubukata K. Prolonged *Mycoplasma pneumoniae* infection in an elderly patient with community-acquired pneumonia. *J Infect Chemother* 2009;**15**:243–7.
8. The Committee for the Guidelines in Management of Respiratory Infectious Diseases in Children. Upper respiratory infection and bronchitis. In: Uehara S, Sunakawa K, editors. *Guidelines for the management of respiratory infectious diseases in children in Japan 2007*. 1st ed. Tokyo: Japanese Society of Pediatric Pulmonology and Japanese Society for Pediatric Infectious Diseases; 2007. p. 29–40.
9. Dabitao D, Margolick JB, Lopez J, Bream JH. Multiplex measurement of proinflammatory cytokines in human serum: comparison of the Meso Scale Discovery electrochemiluminescence assay and the Cytometric Bead Array. *J Immunol Methods* 2011. doi:10.1016/j.jim.2011.06.033.
10. Hara Y, Ohtsu N, Goto K, Watanabe H. Variations of antibody titers and circulating cytokines' levels in human metapneumovirus-infected children. *Kansenshogaku Zasshi* 2009;**83**:566.
11. Jartti T, van den Hoogen B, Garofalo RP, Osterhaus AD, Ruuskanen O. Metapneumovirus and acute wheezing in children. *Lancet* 2002;**360**:1393–4.
12. Huck B, Neumann-Haefelin D, Schmitt-Graeff A, Weckmann M, Mattes J, Ehl S, Falcone V. Human metapneumovirus induces more severe disease and stronger innate immune response in BALB/c mice as compared with respiratory syncytial virus. *Respir Res* 2007;**8**:6.
13. Melendi GA, Laham FR, Monsalvo AC, Casellas JM, Israele V, Polack NR, Kleeberger SR, et al. Cytokine profiles in the respiratory tract during primary infection with human metapneumovirus, respiratory syncytial virus, or influenza virus in infants. *Pediatrics* 2007;**120**:e410–5.
14. Hamelin ME, Yim K, Kuhn KH, Cragin RP, Boukhvalova M, Blanco JC, Prince GA, et al. Pathogenesis of human metapneumovirus lung infection in BALB/c mice and cotton rats. *J Virol* 2005;**14**:8894–903.
15. Hamelin ME, Prince GA, Gomez AM, Kinkead R, Boivin G. Human metapneumovirus infection induces long-term pulmonary inflammation associated with airway obstruction and hyperresponsiveness in mice. *J Infect Dis* 2006;**193**:1634–42.
16. Hamelin ME, Couture C, Sackett MK, Boivin G. Enhanced lung disease and Th2 response following human metapneumovirus infection in mice immunized with the inactivated virus. *J Gen Virol* 2007;**88**:3391–400.
17. Ditt V, Lüsebrink J, Tillmann RL, Schildgen V, Schildgen O. Respiratory infections by HMPV and RSV are clinically indistinguishable but induce different host response in aged individuals. *PLoS One* 2011;**6**:e16314.
18. Corneli HM, Zorc JJ, Mahajan P, Shaw KN, Holubkov R, Reeves SD, Ruddy RM, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. *N Engl J Med* 2007;**357**:331–9.
19. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics* 2006;**118**:1774–93.
20. Pinto RA, Arredondo SM, Bono MR, Gaggero AA, Díaz PV. T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. *Pediatrics* 2006;**117**:e878–86.
21. Somers CC, Ahmad N, Mejias A, Buckingham SC, Carubelli C, Katz K, Leos N, et al. Effect of dexamethasone on respiratory syncytial virus-induced lung inflammation in children: results of a randomized, placebo controlled clinical trial. *Pediatr Allergy Immunol* 2009;**20**:477–85.
22. Wong JY, Rutman A, O'Callaghan C. Recovery of the ciliated epithelium following acute bronchiolitis in infancy. *Thorax* 2005;**60**:582–7.
23. Kanda T, Takahashi T. Interleukin-6 and cardiovascular diseases. *Jpn Heart J* 2004;**45**:183–93.