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ORIGINAL ARTICLE

Viral etiology of acute lower respiratory tract infections in hospitalized young children in Northern Taiwan

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Bocavirus;
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Background: Lower respiratory tract infections (LRTIs) comprise a great proportion of diagnoses among hospitalized children. This study identifies the viral pathogens causing LRTIs in young children and compares their clinical features and disease severity.
Methods: Children younger than 36 months old, hospitalized at a medical center in Northern Taiwan with acute bronchiolitis or pneumonia from April to December 2007, were prospectively enrolled. Nasopharyngeal aspiration fluid samples were sent for virus culture, for direct immunofluorescence test of respiratory syncytial virus (RSV), for rapid influenza viral identification, and for polymerase chain reaction of human metapneumovirus (hMPV), human boca virus (hBoV), and human corona virus. The clinical features and laboratory findings were recorded and analyzed.
Results: A total of 48 children were enrolled. RSV was the most common pathogen (41.7%), followed by hMPV (27.1%), hBoV, and enterovirus (both 6.3%). There were no significant differences in clinical presentation and disease severity between the RSV and hMPV groups. However, the hMPV group had a higher mixed infection rate ($p = 0.038$). Fourteen children had no identifiable viruses. Children with single, dual, and triple pathogens numbered 26, 7, and 1, respectively. The mixed infection rate reached 23.5% among 34 children with identifiable viruses. Children with a higher severity score had greater chance to develop asthma in the next 2 years ($p = 0.042$).

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Conclusion: RSV is the most common pathogen causing LRTIs in young children, followed by hMPV. The hMPV group had higher mixed infection rate than RSV group. hBoV does circulate in northern Taiwan.

Introduction

Lower respiratory tract infection (LRTI) refers to infections of the trachea, bronchus, and lungs, including tracheitis, bronchitis, bronchiolitis, and pneumonia. The most common viral LRTIs among young children are acute bronchiolitis and pneumonia. Acute bronchiolitis is characterized by tachypnea, wheezing, suprasternal or intercostal retraction, sometimes cyanosis or apnea, and even respiratory failure.[1] As a virus-predominant disease, the most common acute bronchiolitis pathogen is respiratory syncytial virus (RSV), causing more than 50% of cases. Other respiratory pathogens include the influenza virus, parainfluenza virus, adenovirus (ADV), rhinovirus, and enterovirus.[2]

Human metapneumovirus (hMPV), human bocavirus (hBoV), and human coronavirus NL-63 (hCoV NL-63) are three of the novel viruses resulting in similar symptoms to RSV, including acute wheezing episodes.[3,4] Their incidences among children with LRTI vary between 1.5—17.5%,[5] 1.5—11.3%,[6] and 1.3—9.3%,[7,8] respectively. Detection of these viruses relies on polymerase chain reaction (PCR) in reference laboratories.

Concomitant detection of two or three respiratory viruses in one individual could occur. Bonzel et al.[9] reported a 21% mixed infection rate among children with virus positive acute respiratory infection. The combination of RSV and hBoV accounts for two-thirds of their mixed infections. The occurrence of dual infection is a risk factor of greater disease severity.[10] Evidence also showed that severe viral infection in infancy or early childhood is related to recurrent wheezing and asthma in later life, especially RSV and hMPV infections.[11]

This study explores the epidemiology of viral LRTIs in young children, including single and mixed infections. We also tried to sketch the clinical picture of novel respiratory viruses compared with RSV bronchiolitis.

Methods

Patients

The inclusion criteria for this study were: (1) young children aged younger than 36 months; (2) hospitalization at general wards or newborn center of Mackay Memorial Hospital, Taipei from April 1 to December 31, 2007; and (3) a diagnosis of acute bronchiolitis and/or pneumonia. Acute bronchiolitis was defined as acute episodes of cough, rhinorrhea, wheezing or rales, with chest X-ray findings of over aeration, peribronchial infiltration with or without atelectasis. Pneumonia was defined as febrile episodes with rales and chest X-ray findings for airbronchogram or haziness of the lung fields. Fully informed consents were acquired and patients whose parents refused to join this project were excluded.

Specimens

We prospectively collected oropharyngeal swabs with sterile cotton buds and nasopharyngeal aspiration (NPA) fluids from all subjects within 48 hours of admission. The specimens were preserved in standard transport media under 4°C refrigeration and were transported to the Department of Clinical Virology and Microbiology Laboratory of our hospital for virus culture, antigen detection, and nucleic acid (NA) amplification. Three to five milliliters of serum was obtained from each subject for complete blood counts and C-reactive protein.

Viral identification

The oropharyngeal swabs were inoculated on four cell lines (MRC-5 from fibroblast of human fetal lung, Hep-2, A549 from laryngeal carcinoma, and RD cell from rhabdomyosarcoma) for isolation of respiratory viruses. The type of viruses was confirmed by reactions with immunofluorescent antibodies following typical cytopathic effects.

NPA fluid samples were sent for direct immunofluorescence assay to detect RSV (IMAGEN RSV, OXOID, Basingstoke, UK) and Influenza A and B (QuickVue Influenza Test, QUIDEL, San Diego, CA, USA).

The NA amplification method was applied to identify the three novel respiratory viruses. NAs were extracted from 200 μL of NPA fluid with High Pure Viral Nucleic Acid Kit (Roche, Basel, Switzerland) and QIAamp Viral RNA Mini Kit (QIAGEN, Duesseldorf, Germany). For hBoV, PCR was performed with 2 primers of 188 forward (GACCTCTG TAAGTACTATTAC) and 542 reverse (CTCTGTGTTGACTGAA TACAG).[12] For hMPV and hCoV NL-63, complementary DNA was synthesized by using 10 μL of eluted RNA and Advantage RT-for-PCR kit (Clontech, Mountain View, CA, USA). PCR for hMPV was performed with two new primers: N1 forward (TCTACAGGCAGCAAAGCAGA) and N1 reverse (TTTGGG CTTTGCCTTAAATG), amplifying a 224-bp region on nucleoprotein (N) gene of hMPV.[13] Nested PCR for hCoV NL-63 was performed with two sets of primers: repSZ-l (GTGATGCA TATGCTAATTTG) and repSZ-3 (CTCTTGCAGGTATAATCCTA), repSZ-2 (TTGGTAAACAAAAGATAACT) and repSZ-4 (TGAATGGTATAAACAGTCAT).[14]

Clinical data

The clinical data of concern included demographic data (age, sex, and underlying disease), history (past history of atopic diseases and LRTIs, symptoms and signs contributory to the diagnosis of bronchiolitis and/or pneumonia, vital signs at admission, and duration of fever), in-hospital workup (complete blood counts, C-reactive protein, and chest X-ray), treatment (type and duration of O_2 support), and outcome (duration of hospitalization and complications).

Table 1 Five-component bronchiolitis clinical score system for rating the severity of acute bronchiolitis[15]

Score	0	1	2
Duration of hospital stay (d)	<3	4—7	>8
Respiratory support	Nil	O_2 tent	Respirator
Wheeze	Inspiratory phase	Inspiratoy and expiratory phase	Diminishing breath sounds
Respiratory rate (/min)	20—40	40—60	>60
Dyspnea			
Nasal flaring/subcostal retraction	Nil	Yes, without cyanosis	Yes, with cyanosis

Further development of asthma within 2 years after current illness according to chart review was recorded. Atopic diseases referred to atopic dermatitis, allergic rhinitis, and asthma. Dyspnea was defined as presence of tachypnea, cyanosis, suprasternal, or intercostal retractions. The severity of acute bronchiolitis was rated by a 5-component bronchiolitis clinical score system, modified from Bentur's[15] study in 1992, ranging from 0 to 10 points (Table 1). Mild, moderate, and severe acute bronchiolitis were defined as total score of 0—3, 4—7, and 8—10 points, respectively.

Statistic analysis

All data were expressed with mean ± standard deviation or median with range. Categorical data were analyzed using χ^2 test or Fisher's exact test. Intervals and ordinal data were analyzed using the Mann-Whitney *U* test. Statistical significance was defined as a *p* value less than 0.05.

Results

A total of 48 children aged 2—32 months (mean 11.6 ± 7.7 months) were enrolled and followed up for at least 2 years (till December, 2009). There were 30 males and 18 females with a male-to-female ratio of 1.6 to 1. The diagnoses were acute bronchiolitis (36 patients, 75%), pneumonia (4 patients, 8.3%) or both (8 patients, 16.7%). Detailed demographic data were showed in Table 2. All patients achieved full recovery without long-term sequelae. According to the chart review, 13 children had newly diagnosed asthma during the follow-up period.

A total of 33 children's specimens underwent both virus culture and PCR procedure. Among them, 11 children had identifiable viruses with virus culture (virus yield rate: 33.3%), whereas an additional 7 children were found to have novel respiratory viruses with PCR methods (overall virus yield rate: 54.5%).

Table 2 Demographic data of patients with acute bronchiolitis or pneumonia

	Patient, *n* (%)	Range	Mean	Standard deviation
Age (mo)		2.1—32.0	11.6	7.7
Gender (male)	30 (62.5)			
Underlying disease				
Pulmonary	4			
Extrapulmonary	2			
Past history				
Asthma	3			
Atopy	6			
Acute bronchiolitis	7			
Duration of hospitalization (d)		3—11	5.2	2.1
Fever	37 (77.0)			
Duration of fever (d)		1—20	3.9	3.4
Highest temperature (°C)		38.0—40.9	39.2	1.0
Dyspnea	33 (68.8)			
O_2 support (O_2 hood)	42 (87.5)			
Duration of O_2 support (d)		1—9	4.5	2.2
White blood cell counts (1,000/μL)		4.7—24.6	11.4	4.2
Eosinophil counts (μL)		0—1,608	170	295
C-reactive protein (mg/dL)		0.01—14.80	1.89	3.11
Bronchiolitis clinical score		0—7	3.5	1
Complication				
Croup	2			
Acute otitis media	9			
Sinusitis	6			
Pneumonia	12 (25.0)			
Newly developed asthma[a]	13 (27.1)			

[a] Asthma was diagnosed during the follow-up period.

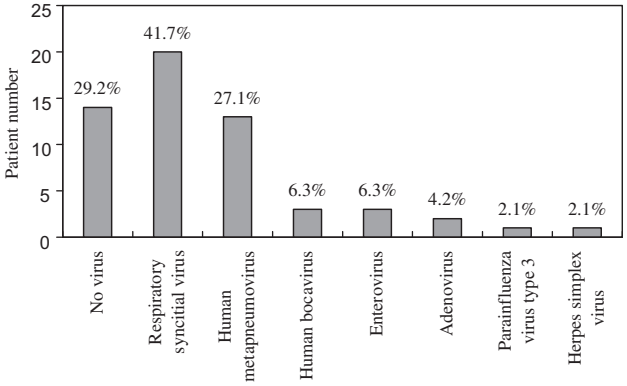

Figure 1. Identified viruses in children with lower respiratory tract infection.

Type of virus

Forty-three respiratory viruses were detected. The two most predominant pathogens were RSV and hMPV (Fig. 1). Comparing the clinical data from these two groups, most variables showed no significant difference (Table 3), except that mixed infection with other respiratory viruses was more common in the hMPV group ($p = 0.038$). There was a trend toward longer O_2 demand in RSV-infected children than hMPV-infected ones ($p = 0.053$). No any hCoV NL-63 or influenza virus was identified in our patients.

Type of infection

The numbers of patients with 0, 1, 2, and 3 kinds of viruses identified were 14, 26, 7, and 1, respectively (Fig. 2). Mixed infection comprised 23.5% of 34 children with identifiable viruses. The clinical features of patients with single and mixed infection are shown in Table 4. Dual infections occurred in RSV with hMPV (two patients), hBoV (one patient), or HSV (one patient), as well as in hMPV with hBoV (one patient), ADV (one patient) or parainfluenza virus3 (one patient). One patient had RSV, hMPV, and ADV identified at the same time.

Correlation between LRTI and asthma

Children with higher bronchiolitis clinical scores had higher chance to have asthma in later life ($p = 0.042$). Its odds ratio for asthma was 4.33 (95% confidence interval = 1.16–16.26).

Table 3 Comparison of clinical data between children with respiratory syncytial virus and human metapneumovirus infection

	RSV ($n = 20$)	hMPV ($n = 13$)	p
Age (mo)	9.2 (2.4–27.7)	11.0 (2.1–32.1)	0.796
Gender			
Male	15	7	
Female	5	6	0.270
Underlying disease	0	1 (ASDII)	
Past history			
Asthma	2	2	1.000
Atopy	4	2	1.000
Acute bronchiolitis	1	1	1.000
Mixed infection	6 (30%)	9 (69%)	0.038
Duration of hospitalization (d)	4.5(3–9)	4 (2–10)	0.267
Fever	16	10	1.000
Duration of fever (d)	4 (1–8)	2.5 (1–6)	0.412
Highest temperature(°C)	39.0 (38.2–40.9)	39.6 (38.7–40.0)	0.969
Dyspnea	16	8	0.425
O_2 support (O_2 hood)	19	10	0.276
Duration of O_2 support (d)	4 (0–9)	2 (0–5)	0.053
Respiratory rate (/min)	32 (25–56)	30 (22–50)	0.454
Heart rate (/min)	134 (114–156)	140 (116–160)	0.985
White blood cell counts (1,000/μL)	9.25 (5.0–18.8)	9.4 (4.7–16.6)	0.580
Eosinophil counts (μL)	36 (0–564)	58 (0–332)	0.830
C-reactive protein (mg/dL)	0.52 (0.04–9.01)	0.35 (0.01–2.21)	0.417
Hemoglobin (g/dL)	12.0 (10.1–14.1)	11.5 (9.2–12.9)	0.209
Platelet (K/μL)	306 (169–495)	409 (184–678)	0.074
Bronchiolitis clinical score	4 (2–5)	3 (0–5)	0.151
Mild (0–4)	14	11	
Moderate (5–8)	6	2	0.431
Associated condition			
Acute otitis media	3	1	1.000
Sinusitis	3	1	1.000
Pneumonia	5	3	1.000
Newly developed asthma[a]	5	5	0.461

[a] Asthma was diagnosed during the follow-up period.
ASDII = Atrial septal defect type II; hMPV = human metapneumovirus; RSV = respiratory syncytial virus.

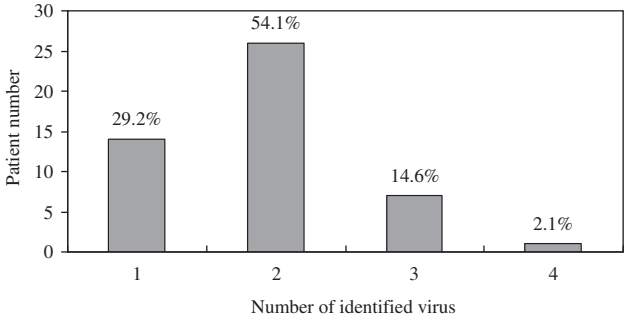

Figure 2. Numbers of identified virus in children with lower respiratory tract infection.

However, the odds ratios of RSV and hMPV infection to asthma in later life were 0.52 (95% confidence interval = 0.15—1.83) and 1.36 (95% confidence interval = 0.36—5.13), respectively.

Discussion

With the development of PCR techniques, novel respiratory viruses are being found, allowing us to better explore the picture of acute respiratory tract infections. So far, PCR-based surveys on the epidemiology of young children LRTIs in Taiwan have been limited.[7,16,17] The present study described the incidence, demographic characteristics, and clinical characteristics of LRTIs caused by various viruses. In one recent PCR-based study on children below 2 years old with acute bronchiolitis, the viral isolation rate elevated from 48% to 90% after applying PCR technique in addition to traditional virus culture.[2] Another study enrolled 182 infants hospitalized with acute bronchiolitis showed a rise in virus yield rate from 42.3% to 57.2%.[18] In our study, the virus yield rate was 33.3% with virus culture and 54.5% with additional PCR methods. These results suggest that PCR technique is a promising tool in clinical virology studies, helping researchers to explore viral diseases in a more comprehensive perspective.

As in the previous literature, RSV is always the leading pathogen of acute bronchiolitis. The isolation rate varies with different study designs and geographic areas. Mlinaric-Galinovic et al.[18] reported that RSV was responsible for 49% of 1,010 hospitalized Croatian infants with LRTI and younger than 6 months old over an 11-year period. In the study of Miron et al.[2] in Israel, 490 previously healthy babies younger than 24 months hospitalized for acute bronchiolitis during 4 winter months were enrolled. RSV was identified in 76% of children. Midulla et al.[19] found RSV in 41.2% of 182 Italian infants with acute bronchiolitis in three consecutive annual epidemic periods. In our study, RSV was the most frequently

Table 4 Comparison of clinical data between children with single and mixed infection

	Single infection (n = 26)	Mixed infection (n = 8)
Age (mo)	11.2 (4.1—18.3)	13.8(5.1—22.6)
Gender		
Male	21	3
Female	5	5
Past history		
Asthma	3	1
Atopy	4	2
Acute bronchiolitis	3	0
Duration of hospitalization (d)	5.2 (3.1—7.4)	3.8 (2.9—4.6)
Fever	21	7
Duration of fever (d)	3.4 (1.2—5.6)	3.4 (1.9—4.9)
Highest temperature (°C)	39.2 (38.3—40.0)	39.3 (38.9—39.7)
Dyspnea	20	4
O_2 support (O_2 hood)	25	6
Duration of O_2 support (d)	4.4 (2.1—6.6)	2.5 (0.5—4.5)
Respiratory rate (/min)	37 (26—47)	36 (27—44)
Heart rate (/min)	135 (122—147)	132 (122—146)
White blood cell counts (1,000/μL)	10.4 (6.2—14.5)	10.9 (6.8—15.0)
Eosinophil counts (/μL)	178 (0—498)	48 (0—114)
C-reactive protein (mg/dL)	1.65 (0—4.06)	0.82 (0—1.67)
Hemoglobin (g/dL)	11.8 (10.6—13.0)	11.6 (11.0—12.2)
Platelet (K/μL)	315 (229—402)	415 (279—550)
Bronchiolitis clinical score	3.8 (2.8—4.8)	2.6 (0.6—4.6)
Mild (0—4)	17	7
Moderate (5—8)	9	1
Associated condition		
Acute otitis media	6	0
Sinusitis	4	0
Pneumonia	5	3
Newly developed asthma[a]	7	3

[a] Asthma was diagnosed during the follow-up period.

detected virus, presented in 41.7% of all patients. RSV in Taiwan circulates year-round and peaks mainly in spring and fall with annual variation.[20] Thus, if we enrolled patients over 12 months the incidence of RSV may change.

hMPV isolation has been reported around the world since 2001,[21,22] with various prevalence from 1.5% to 17.5% in children hospitalized with acute respiratory illness. However, hMPV-positive patients accounted for up to 27.1% in our study, which may be related to N gene use with higher sensitivity in hMPV detection. Regarding the difference between RSV and hMPV, Chan et al. reported that RSV-infected children at a younger age lead to longer hospital stays and higher oxygen demand than hMPV did.[23] On the other hand, Viazov reported that the clinical characters of RSV and hMPV bronchiolitis showed no significant difference, except the duration of symptoms was shorter in hMPV-infected patients.[24] Mullins' data showed similar disease severity in RSV and hMPV-infected children.[25] No significant difference was found in hospital stay or fever duration, severity score, white blood cell counts, C-reactive protein, or complication rate in our study, similar to Carraciolo's report.[26] However, RSV-positive patients showed a trend for longer duration of O_2 demand ($p = 0.053$). The discrepancy among these studies may result from the great heterogenecity in studied populations (age group, diagnosis, and inpatient/outpatient). To differentiate these two viruses judging by clinical information is difficult. Therefore, further researches with large sample sizes and detailed subgroup analyses are necessary.

The prevalence of hBoV varies considerably between 1.5 and 11.3% in children suffering from acute respiratory infections.[6] In addition to LRTIs, it can cause wheezing, respiratory distress, hypoxia, fever, rhinitis, and laryngeal croup in children.[27] A high rate of coinfection with other respiratory viruses (up to 91% in Thailand[6]) makes it difficult to clarify the pathogenic role of hBoV. In the Italian report, hBoV was isolated in 8.2% of patients, including 61.8% of coinfection with various viruses, which resulted in difficulties in defining the true causative virus.[28] Dina et al.[29] reported a 1.6% incidence of hBoV in hospitalized children in France, with coinfection presented in four of seven hBoV-positive children. The prevalence rate of hBoV in our study was 6.25%, and two of the three hBoV-positive patients had more than one pathogen identified (coinfected with RSV and hMPV, respectively). This result suggests that hBoV does circulate in our community.

hCoV NL-63 has been reported to play a role in acute respiratory tract infection in infants and children, with a percentage from 1.3% in Taiwan[7] and up to 9.3% in France.[8] It is more likely to present as croup or acute bronchiolitis,[30] but pharyngitis, rhinitis, otitis, conjunctivitis, and pneumonia are all within its clinical spectrum. The present study failed to identify any one patient with hCoV NL-63, which may result from its relative low incidence and small sample size.

Simultaneous existence of more than one virus in an individual patient has been reported, with a mixed infection rate ranges from 10% to 30% among hospitalized children, mostly RSV-hMPV and RSV-rhinovirus.[9,19,26,31] In the present study, the mixed infection rate was 16.7% (8 of 48 patients). Furthermore, 30% of RSV-positive (6 of 20) and 69% of hMPV-positive (9 of 13) patients had more than one virus identified. The rate of mixed infection is significantly higher in hMPV group ($p = 0.038$). In previous literatures, the frequency of hMPV and RSV coinfection ranges from 0 to 20%.[32] Whether a mixed infection causes more severe disease is still controversial. Some reported that simultaneous infection with RSV and hMPV was related to higher severity,[10] whereas the other did not.[32] We found no significant difference in severity (duration of hospitalization, fever, O_2 support, and severity score) among RSV, hMPV, and RSV/hMPV groups. A larger population is needed for clarifying the correlation between dual infection and disease severity.

It is reported that severe viral respiratory infections are more likely to have asthma later in childhood.[11] We found that high severity score is a risk factor of asthma with an odds ratio of 4.33. Although limited data were not sufficient to identify the correlation between individual viral pathogen and asthma, it seemed to be certain of the severe LRTIs effect on the development of asthma. Long-term follow-up and expansion to intensive care unit patients are necessary to get more concrete conclusion.

There are a few limitations in our study. The small-sized population lowers the probability of catching statistical difference and precludes detailed subgroup analysis. A relatively short study period impedes observation of seasonality and may affect the distribution of viruses. Because not many influenza infections occurred in Taiwan during the study period, it could explain why we did not detect the influenza virus. The exclusion of outpatients and intensive care unit patients leads to a conclusion applied to a specific range of disease severity.

In conclusion, with limited PCR-based studies on viral respiratory tract infections in Taiwan, the present study pictures viral epidemiology in young hospitalized children with the focus on novel respiratory viruses. RSV is the most common as a single pathogen, followed by hMPV, which had significantly higher rate of coinfection compared with RSV. Otherwise, the clinical characteristics of hMPV do not significantly differ from those of RSV. Mixed infection was noted in 23.5% of patients with identifiable viral pathogens. hBoV does circulate in the population of Northern Taiwan.

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