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## Research Article

### Detection of Cytomegalovirus Nucleic Acid and Mycoplasma Nucleic Acid in Alveolar Lavage Fluid of 31 Children with Respiratory Tract Infection

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#### Abstract

**Objective:** To investigate the infection of cytomegalovirus and *Mycoplasma pneumoniae* in alveolar lavage fluid of children with respiratory tract infection.

**Methods:** A total of 31 children with respiratory tract infection were enrolled in the Department of Pediatrics, the First Affiliated Hospital of Anhui Medical University from May to August in 2017, and the cytomegalovirus nucleic acid in the alveolar lavage fluid was detected by real-time fluorescent polymerase chain reaction. Ribonucleic Acid (RNA) thermostatic amplification technology was used to detect *Mycoplasma pneumoniae* nucleic acid in the alveolar lavage fluid.

**Results:** The total detection rate of both pathogens was 64.52%, the positive rate of cytomegalovirus nucleic acid was 38.71%, and the positive rate of *Mycoplasma pneumoniae* was 25.81%.

**Conclusion:** Cytomegalovirus and *Mycoplasma pneumoniae* have high infection rate in children with respiratory tract infection. The combined detection of these two infection agents in alveolar lavage fluid has important application value for clinical etiology and treatment

**Keywords:** Alveolar Lavage Fluid; Cytomegalovirus Nucleic Acid; *Mycoplasma pneumoniae* Nucleic Acid; Respiratory Tract

#### Introduction

Respiratory infection is a common disease in childhood. It can cause bronchitis, pneumonia and other illnesses. As a disease with long course, it has serious impact on children's growth and development. The infectious pathogens have various types, including bacteria, viruses and mycoplasma, and the etiology is complex. In recent years, due to the migration of pathogens, Human Cytomegalovirus (HCMV) and *Mycoplasma pneumoniae* (MP) infection showed a rising trend. With the development of molecular diagnostic technique and the improvement of clinical specimen collection technology, the joint detection of pathogens gradually attracted more attention [1-2]. This paper examined the joint detection of HCMV nucleic acid and MP nucleic acid in 31 children with respiratory tract infection from May to August 2017 in our hospital.

#### Material and Methods

##### Study Participants

Participants were recruited at the first affiliated hospital of Anhui Medical University from May to August 2017, a total of 31 children with respiratory tract infections. Study protocol was approved by the Institutional Review Board.

There were 19 male, 12 female, 12 patients with age 1-12 years, 14 patients 1-3 years old, 8 patients 4-6 years old, and 9 patients 7-12 years old. Based on radiology assessment, 9 patients were diagnosed as bronchitis, and 22 as pneumonia. Patients were divided into 3 age groups: 14 infants (1-3 years, 45.16%), 8 toddlers (>3-6 years, 25.81%), 9 children (> 6 years, 29.03%).

##### Experimental Procedures

Alveolar lavage fluid was collected as part of the clinical procedure. HCMC nucleic acid was detected by real-time fluorescent Polymerase Chain Reaction (PCR) with cytomegalovirus nucleic

acid quantitative detection kit (Da An, Zhong Shan, Guang Dong, China), and Ribonucleic Acid (RNA) thermostatic amplification technology was used to detect MP nucleic acid with MP nucleic acid detection kit (Ren Du, Shanghai, China) on real-time fluorescence quantitative PCR instrument Roche Cobas z480 (Roche Diagnostics, Basel, Switzerland).

## Results

### Etiology Analyses

A total of 20 cases of HCMV positive and MP positive were detected (detection rate 64.52%). HCMV was positive in 12 cases (38.71%), and MP was positive in 8 cases (25.81%). There were two cases with mixed infection (6.45%).

### Detection Rate of Two Pathogens in Children of Different Age Groups

As shown in (Table 1), the highest positive rate of HCMV infection was found in infants (50.00%), MP positive infection rate in toddlers was the highest (25.00%). However, the detection rate of in each age group was not statistically significant from each other (HCMV  $\chi^2 = 3.193$ ,  $P = 0.203$ ; MP  $\chi^2 = 2.606$ ,  $P = 0.272$ ).

Age (years)	Cases (n)	HCMV positive (n/%)	MP positive (n/%)
3-Jan	14	7 / 50.00	2 / 14.29
>3-6	8	1 / 12.50	2 / 25.00
>6	9	4 / 44.44	4 / 12.90
$\chi^2$	-	3.193	2.606
P	-	0.203	0.272

**Table 1:** Comparison of two pathogen detection rates in 31 cases of children in different age groups.

### Detection Rate of Two Pathogens in Different Months

As shown in (Table 2), the positive rate of HCMV infection was the highest in May and August (both 80.00%) and the positive rate of MP infection was the highest in June (37.50%).

However, the detection rate of in each month was not statistically significant from each other (HCMV  $\chi^2 = 2.890$ ,  $P = 0.272$ ; MP  $\chi^2 = 0.798$ ,  $P = 0.839$ ).

Month	Cases (n)	HCMV positive (n/%)	MP positive (n/%)
5	5	4 / 80.00	1 / 20.00
6	8	5 / 62.50	3 / 37.50
7	13	10 / 76.92	3 / 23.08
8	5	4 / 80.00	1 / 20.00
$\chi^2$	-	2.89	0.798
P	-	0.272	0.839

**Table 2:** Comparison of two pathogen detection rates in different months.

## Clinical Manifestations

There were symptoms of coughing in 27 cases, fever in 12 cases, lung-positive signs in 21 cases, hyperpnea in 6 cases, wheeze in 5 cases, fine wet rales in 6 cases, phlegm in 3 cases, sibilant rhonchi in 1 case. In laboratory tests, white blood cell counts in 22 patients were normal, 8 patients with counts of  $> 10 \times 10^9$ , 1 patient with  $4 \times 10^9$ . Among the 20 patients that were pathogen positive, 9 cases had bronchitis, 22 cases had pneumonia and 7 cases had electrocardiogram: 4 Cases of sinus tachycardia, and 3 cases of sinus arrhythmia. Hospitalization duration ranged from 3 to 17 days, of which length of HCMV infection stay was  $10.44 \pm 4.22$  days (mean  $\pm$  standard deviation), MP infection was  $10.13 \pm 1.89$  days, the virus-negative cases had duration of  $8.71 \pm 3.41$  days.

## Discussion

Most of the studies on pathogens of respiratory tract infection in children focused on bacteriological or virological studies, and few reports on the mixed infection of common atypical pathogens like MP and HCMV viruses. In this study, we collected pulmonary alveolar lavage fluid from 31 pediatric patients with respiratory tract infection from May to August 2017. The prevalence of HCMV and MP pathogens were investigated by real-time fluorescence PCR and RNA thermostable amplification. The results showed that the total detection rate of these two pathogens was as high as 64.52%, the detection rate of HCMV (38.71%) was higher than that of MP (25.81%). Two patients had the two pathogens detected simultaneously (6.45%). The positive rate of HCMV infection was the highest in infants (50.00%), and the positive rate of MP infection was the highest in toddlers (25%). The positive rate of HCMV infection was the highest in May and August (80.00%). The positive rate of MP was the highest in June (37.5%). The detection rates of pathogens in children of different ages and months were different, but the difference was not statistically significant. It was confirmed that HCMV and MP were both detected in children with respiratory tract infections with a high infection rate, mainly HCMV.

HCMV and MP are the most common pathogens of viral infection in children and immune-deficient patients. They can affect various organs of the body. In particular, pulmonary infections are more common and the condition is more serious. HCMV and MP cause pneumonia but sometimes clinical manifestations and radiology assesment lack specificity. The diagnosis is confirmed by bronchial alveolar lavage fluid pathogen or bronchoscopy lung biopsy or open lung biopsy. Although all of the above three procedures are invasive, patients tend to have higher acceptance to alveolar lavage than the other two. The positive detection rates in previous literature reports vary, possibly due to etiology of respiratory viruses and differences in races, regions, years, seasons, age and other factors [3-4]. We took the alveolar lavage fluid as research subject, using real-time fluorescence PCR method to detect HCMV, and the new technology of RNA thermostat amplification

to detect MP. Real-time fluorescence PCR is a classical clinical nucleic acid detection method, while RNA thermostat amplification is a new nucleic acid detection technology combining nucleic acid thermostat amplification with real-time fluorescence detection [5]. The target and amplification products are RNA, with higher sensitivity and specificity, and using magnetic bead-based specific extraction of nucleic acids. In addition, since RNA degrades rapidly, this method allows “dead pathogens” and “live pathogens” to be distinguished. The results are clinically relevant and provide important guidance for clinical judgment by physicians [6]. The two methods used in this study are sensitive and contribute to the early diagnosis of pathogens and provide an early basis for treatment to prevent damage to other vital organs.

## References

1. Cinel G, Pekcan S, Ozçelik U, Alp A, Yalçın E, et al. (2014) Cytomegalovirus infection in immunocompetent wheezy infants: the diagnostic value of CMV PCR in bronchoalveolar lavage fluid. *J Clin Pharm Ther* 39: 399-403.
2. Burgener EB, Waggoner J, Pinsky BA, Chen SF (2017) Clinical characteristics and outcomes of pediatric patients with CMV DNA detection in bronchoalveolar lavage fluid. *Pediatr Pulmonol* 52: 112-118.
3. Li W, Fang YH, Shen HQ, Yang DH, Shu Q, et al (2017) Evaluation of a real-time method of simultaneous amplification and testing in diagnosis of *Mycoplasma pneumoniae* infection in children with pneumonia. *PLoS One* 12: e0177842.
4. Inchley CS, Berg AS, Vahdani Benam A, Kvissel AK, Leegaard TM, et al. (2017) *Mycoplasma pneumoniae*: A Cross-sectional Population-based Comparison of Disease Severity in Preschool and School-age Children. *Pediatr Infect Dis J* 36: 930-936.
5. Chen Y, He H, Pan P, He S, Dong X, et al. (2016) Rapid and combined detection of *Mycoplasma pneumoniae*, Epstein-Barr virus and human cytomegalovirus using AllGlo quadruplex quantitative PCR. *J Med Microbiol* 65: 590-595.
6. Li W, Fang YH, Shen HQ, Yang DH, Shu Q, et al. (2017) Evaluation of a real-time method of simultaneous amplification and testing in diagnosis of *Mycoplasma pneumoniae* infection in children with pneumonia. *PLoS One* 12: e0177842.