



LETTER

## Retrospective Study of an Adenovirus Pneumonia Outbreak in Shenzhen in 2017

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Dear Editor,

Human adenoviruses (HAdVs) are associated with respiratory, gastrointestinal, ophthalmological, genitourinary, and neurological infections. To date, more than 100 genotypes of HAdVs have been identified. They are classified into seven species, A to G (HAdV Working Group, <http://hadvvg.gmu.edu/>). HAdVs associated with lower respiratory tract infection endanger children's health due to their poor prognosis, high mortality, severe complications, and serious sequelae (Hong *et al.* 2001).

In 2012, an outbreak of adenovirus pneumonia occurred in Shenzhen. During that period, HAdV-B7 and HAdV-B3 were identified as dominant types and the children's symptoms were generally mild (Wang *et al.* 2015). In 2017, another outbreak of adenovirus pneumonia with more severe symptoms occurred in Shenzhen. To increase physicians' awareness of adenovirus pneumonia and improve clinical practice, we reviewed the clinical characteristics of adenovirus pneumonia among children in the 2017 Shenzhen outbreak.

Fifty-seven children diagnosed with adenovirus pneumonia between April and July 2017 were included in this study. All patients had fever and coughing. Twenty-eight patients were diagnosed with mild pneumonia, the other 29 patients were diagnosed with severe pneumonia. Severe adenovirus pneumonia was more common in patients with the following characteristics: (1) age  $\leq 2$  years old; (2) higher level of C-reactive protein (CRP) and procalcitonin (PCT); (3) atelectasis in chest computerized tomography (CT); (4) presence of extrapulmonary complications (Table 1).

Nasopharyngeal (NP) secretions of all the patients were collected and tested for antigens of adenovirus, respiratory syncytial virus, parainfluenza virus (type 1, 2, 3), and influenza virus (type A and B) using a D3® Ultra™ DFA (direct fluorescent antibody) Respiratory Virus Screening and Identification Kit (Diagnostic Hybrids, Inc. US). The NP specimens of 28 mild pneumonia patients were tested positive for adenovirus. Bronchoscopy and adenovirus typing were not performed in any of the patients. The other 29 patients diagnosed with severe pneumonia were tested negative for adenovirus and underwent bronchoalveolar lavage (BAL). Metagenomics next-generation sequencing (mNGS) and DFA respiratory virus screening were further performed in all the bronchoalveolar lavage fluid (BALF) specimens.

In mNGS analysis, nucleic acid (including DNA and RNA) was extracted directly from BALF using the TIA-Namp DNA/RNA Isolation Kit (DP422, Tiangen Biotech) in accordance with the manufacturer's standard protocols. Agilent 2100 was used for quality control of the DNA libraries, which were sequenced on BGISEQ-100 platform. All sequencing reagents (PMSEQ) and PMseq software were approved by China Drug Administration (CDA). The sequencing data were deposited in the GenBank database under accession number PRJNA572371. Processed by removing low-quality and short (length  $< 35$  bp) reads, sequencing data then were aligned with the human reference genome (hg19) to remove human-derived sequences using Burrows–Wheeler Alignment. The remaining data were classified by simultaneously aligning to four Microbial Genome Databases (<ftp://ftp.ncbi.nlm.nih.gov/genomes/>), which contains 4152 whole-genome sequence of viral taxa, 3446 bacterial genomes or scaffolds, 206 fungi related to human infection, and 140 parasites associated with human diseases. The number of unique alignment reads was calculated and standardized to get the number of reads stringently mapped to pathogen species (SDSMRN) and the number of reads stringently mapped to pathogen genus (SDSMRNG). In case it was regarded as likely

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**Table 1** Clinical analysis of all adenovirus pneumonia cases.

Clinical data	Variables	Number (n = 57)		Severe pneumonia (n = 29) (%)	$\chi^2$	P values	
		n	%				
General information	Sex	Male	39	19	48.7	0.230	0.631
		Female	18	10	55.6		
	Age, years	> 2	31	12	38.7	4.026	0.045
		≤ 2	26	17	65.4		
Clinical manifestations	High fever	Yes	57	29	50.9	NA	NA
		No	0	0			
	Breathing	Yes	13	9	69.2	2.270	0.132
		No	44	20	45.5		
Laboratory findings	Leukocytosis	Yes	30	18	54.5	1.133	0.287
		No	27	11	40.7		
	Elevated hsCRP	Yes	31	20	64.5	5.058	0.025
		No	26	9	34.6		
	Elevated PCT	Yes	33	21	63.6	5.105	0.024
		No	24	8	33.3		
Radiological findings	Multifocal infiltrates	Yes	35	20	57.1	1.424	0.233
		No	22	9	40.9		
	Atelectasis	Yes	11	9	81.8	5.221	0.022
		No	46	20	43.5		
	Pleural effusion	Yes	12	7	58.3	0.066	0.798
		No	45	22	48.9		
Underlying disease		Yes	5	3	60.0	0.183	0.669
		No	52	26	50.0		
Complications		Yes	15	11	73.3	4.108	0.043
		No	42	18	42.9		

CRP, C-reactive protein; PCT, procalcitonin.

causative pathogen, pathogen-specific sequence was further verified by Sanger sequencing (Yao *et al.* 2016; Han *et al.* 2013). Adenovirus was detected by mNGS in 29 BALF specimens and detected by DFA respiratory virus screening in 11 BALF specimens. HAdV-B7 and HAdV-B3 were detected in 26 and 3 patients, respectively (Table 2). No other viruses or clinically significant bacteria were detected by mNGS, suggested that all the 29 patients with severe pneumonia were not co-infected with other pathogens.

During the outbreak, all the patients received antibiotic treatment before and after hospitalization. After the viral

infection was confirmed, patients received mezlocillin sodium and sulbactam sodium to prevent bacterial infection and intravenous methylprednisolone (2 mg/kg/d) for anti-inflammation. When body temperature returned to normal, the medicine was administered orally and tapered off based on the clinical manifestations. Bronchoscopy showed widespread obstruction of the airway by mucus in nine patients. And tree-like plastic casts were removed from the bronchi of those patients. Histopathological examinations showed fibrinous exudation and inflammatory cells, consistent with type I plastic bronchitis (PB). Based on the presence of PB, 29 patients diagnosed with

**Table 2** The detection of adenovirus based on DFA respiratory virus screening and mNGS sequencing methods.

Classification of disease	Specimen type	Number	DFA positive	mNGS positive
Mild cases	Nasopharyngeal	28	28	–
Severe cases	Nasopharyngeal	29	0	–
	Bronchoalveolar lavage fluid	29	11	29

DFA, direct fluorescent antibody; mNGS, metagenomics next-generation sequencing; –, not implemented.

severe pneumonia were further divided into a PB group ( $n = 9$ ) and non-PB group ( $n = 20$ ). Patients in the PB group had a shorter duration of fever and hospitalization than those in the non-PB group. Chest CT images usually showed segmental atelectasis with pleural effusion in the PB group and bilateral diffuse ground-glass opacity in the non-PB group (Table 3). In addition, five patients were admitted to the pediatric intensive care unit, among whom three received mechanical ventilation, and one child developed acute respiratory distress syndrome (ARDS) and required extracorporeal membrane oxygenation (ECMO) support. During the 1-year follow-up period, 3 patients developed bronchiolitis obliterans, and no patient died. Furthermore, 14 patients developed extrapulmonary complications, including 2 cases of pericardial effusion, 1 of conjunctivitis, 2 of encephalitis, 3 of enteritis, and 6 of hepatitis.

Adenovirus pneumonia is a common viral pneumonia in children, and it has seasonal and epidemic characteristics. In the outbreak studied here, the peak outbreak occurred between April and July, which was consistent with our previous observations. In China, HAdV-B7 and HAdV-B3 are dominant virus types and HAdV-B7 infections are more severe (Fu *et al.* 2019). In this study, pathogens detected by mNGS in the BALF were mainly HAdV-B7, which was consistent with our previous monitoring results (Wang *et al.* 2015). In contrast, HAdVs causing severe pneumonia in northern China were mainly HAdV-C2 and HAdV-B14 (Yao *et al.* 2019; Yu *et al.* 2012). The difference may be related to the different geographical locations, climates, populations, and detection techniques. The sensitivity of mNGS was higher than that of DFA used for the detection of adenovirus, indicate the importance of the use of a nucleic acid-based test in the diagnosis of the causative pathogens.

When pneumonia causes patients' condition to deteriorate rapidly, with radiological examination showing atelectasis and pleural effusion, physicians should consider PB. PB is characterized by the formation of thick,

obstructive gelatinous casts in the tracheobronchial airway. Patients with PB are usually in critical and rapidly deteriorating condition. PB may be fatal if not treated promptly. Currently, the pathogenesis of PB is poorly understood. In the United States, PB is most common in patients with congenital heart disease, particularly in those who have undergone the Fontan procedure (Rubin 2016). In China, PB is most common in patients with infectious disease, which is distinctively different from reports of other countries. Pathogens commonly associated with PB in China include mycoplasma pneumoniae, influenza virus, and adenovirus (Deng *et al.* 2010; Lu and Zheng 2018). In this adenovirus pneumonia outbreak, BAL was performed in 29 pediatric patients and PB was found in 9 patients. The number of PB patients was unusually higher than it had been before. One possible explanation is that a large number of sick children were hospitalized in a short period of time during the outbreak, and adenovirus pneumonia cases had been mostly sporadic in the past. Other explanations include different adenovirus types, misdiagnosis, missed diagnosis, and limitations of detection in the past.

The use of antibiotics in adenovirus pneumonia is still under debate, mainly because it is difficult to distinguish secondary bacterial infections from single adenovirus infection. Studies have shown that the neutrophil count, CRP, and PCT levels are significantly higher in children with severe adenovirus pneumonia (Kawasaki *et al.* 2002), which suggests that severe adenovirus pneumonia is closely associated with immune response. It has been reported that PCT is better than CRP at differentiating bacterial infection from adenovirus infection in children (Elenius *et al.* 2012). Recent studies found that IL-6 concentration and erythrocyte sedimentation rate are specifically associated with the severity of HAdV-B7 respiratory infection and that infection can also result in a marked increase in CRP and PCT levels (Sun *et al.* 2018). In this study, we obtained similar results. CRP and PCT were significantly higher in children with severe pneumonia than in children with mild pneumonia. In summary, it is very difficult to

**Table 3** Clinical and imaging characteristics of PB and non-PB in 29 severe adenovirus pneumonia cases.

Variables	Total (n = 29)	PB (n = 9)	Non-PB (n = 20)	P values
<i>Clinical features</i>				
Fever days at admission	7 (2, 22)	6 (5, 9)	7 (2, 22)	0.9
Duration for fever after admission, days	6 (0, 21)	4 (2, 7)	8 (0, 21)	0.03
Total febrile days	12 (8, 30)	10 (9, 13)	15 (8, 30)	0.04
Hospitalization days	11 (5, 45)	9 (7, 12)	13 (5, 45)	0.01
<i>Radiological findings</i>				
Pleural effusion	7 (24.1%)	5 (55.6%)	2 (10.0%)	0.01
Atelectasis	9 (31.0%)	7 (77.8%)	2 (10.0%)	0.00
Multifocal infiltrates	20 (69.0%)	4 (44.4%)	16(80.0%)	1.0

Data are presented as median (interquartile range) and n (%).

differentiate between a strong inflammatory response and a secondary bacterial infection in adenovirus pneumonia.

In the severe pneumonia group, all patients received antibiotics, IV methylprednisolone, and BAL. PB patients had shorter duration of fever and hospitalization than non-PB patients. This may be explained by the difference in infected areas of lungs. In PB patients, chest images mainly showed unilateral pulmonary infection with segmental atelectasis. In non-PB patients, chest images mainly showed bilateral diffusive abnormalities and it is difficult to completely remove endotracheal necrotic secretions via BAL. Therefore, for patients with severe adenovirus pneumonia, especially those who develop PB, early BAL is an effective and safe treatment that could shorten the course of the disease.

With the improvement of medical care, the mortality rate of adenovirus pneumonia has decreased significantly. However, the incidences of sequelae, especially bronchiolitis obliterans (BO) and bronchiectasis, remain high. To date, the role of glucocorticoids in the management of adenovirus pneumonia is still unclear. A study in 2006 suggested that glucocorticoids may increase the incidence of BO (Castro-Rodriguez *et al.* 2006). Another study suggested that high-dose methylprednisolone treatment of severe adenovirus pneumonia could achieve better results (Takahashi *et al.* 2006). In this study, 29 patients with severe adenovirus pneumonia were treated with antibiotics, methylprednisolone, and BAL. No patient died. During the 1-year follow-up period, 3 (10.3%) patients with severe adenovirus pneumonia developed BO, which is lower than the previously reported incidence of 14%–60% (Hong *et al.* 2001). This is probably because we performed BALs and used IV methylprednisolone to inhibit airway inflammation and infiltration. However, we had only a limited number of patients in this study. Large prospective studies are needed to validate our hypothesis.

In summary, during the outbreak of adenovirus pneumonia in Shenzhen, the epidemic seasons were spring and summer, and the dominant type of HAdV in severe pneumonia was HAdV-B7. When chest radiological examination shows atelectasis and pleural effusion, physicians should consider the likelihood of PB. BAL performed promptly could reduce the duration of fever and hospitalization. However, large-scale prospective clinical studies are needed to determine if BAL combined with IV methylprednisolone could reduce the mortality rate and incidence of sequelae in children with severe adenovirus pneumonia.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Animal and Human Rights Statement** The study was approved by the Ethics Committee of ShenZhen Children's Hospital. Informed consent was obtained from all patients for the collection and use of all clinical specimens.

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