Adenovirus Viremia Predicts Adenovirus Pneumonia Severity in Immunocompetent Children

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**Abstract**

**Background:** Previous studies have demonstrated an association between adenovirus viremia and disease severity in immunocompromised children. However, few studies focused on the use of this approach in immunocompetent children. This study explored the association between adenovirus viremia and adenovirus pneumonia severity in immunocompetent children.

**Methods:** We did a retrospective, observational study of immunocompetent children with adenovirus pneumonia admitted in Shenzhen Children's hospital in Shenzhen, China. Pneumonia was classified as severe or mild, based on the Chinese guideline of pneumonia severity classification. The serum of all the children in the study was tested for adenovirus DNA with quantitative polymerase chain reaction (PCR). Clinical manifestations, laboratory examinations, and disease severity were compared between these two groups.

**Results:** A total of 111 immunocompetent children with adenovirus pneumonia (60 severe, 51 mild) were included. The median age was 40 months and 64 patients were male. Five patients were admitted to intensive care unit and two were endotracheal intubated. All the patients were discharged with recovery or improvement. Univariate analysis and binary logistic regression analysis showed leukocytosis (OR = 1.1; 95% CI: 1.0 to 1.2; \( P = 0.033 \)), co-infection of mycoplasma pneumoniae (OR = 5.0; 95% CI: 2.1 to 12.3; \( P < 0.001 \)), and high blood viral load (OR = 1.5; 95% CI: 1.2 to 2.0; \( P = 0.001 \)) were risk factors for severe adenovirus pneumonia.

**Conclusions:** Leukocytosis, co-infection of mycoplasma pneumoniae, and high blood viral load are risk factors for severe adenovirus pneumonia in immunocompetent children. Blood viral load predicts pneumonia severity.

**Background**

Early in 1998, a retrospective study of disseminated adenovirus disease in immunocompromised and immunocompetent children found that viremia and prolonged viral excretion were more common in the immunocompromised, though clinical features and outcome were similar [1]. Since 2001, quantitative polymerase chain reaction (PCR) has been wildly used to detect adenovirus genome in blood in immunocompromised children [2]. Previous studies have demonstrated an association between adenovirus viremia and the risk of both disseminated disease and mortality [3–5]. However, few studies explored the role of adenovirus viremia in immunocompetent children.

In 2019 June, a guideline for diagnosis and treatment of adenovirus pneumonia in children was published in China due to an outbreak of human adenovirus [6]. Increased number of immunocompetent children with severe adenovirus pneumonia were observed. Possible risk factors for severe adenovirus pneumonia including viremia were presumed based on studies of immunocompromised children with adenovirus infection, but not confirmed [3–5]. Little was known about the role of adenovirus viremia in immunocompetent children with adenovirus pneumonia.
In this study, we reported clinical characteristics of adenovirus pneumonia in immunocompetent children and explored the role of adenovirus viremia. We also identified risk factors for severe adenovirus pneumonia in immunocompetent children.

Methods

Study design

This study was a retrospective, observational study conducted in Shenzhen Children's Hospital, a 1300-bed tertiary care facility in Shenzhen, China. The study population consisted of all consecutive patients with acute respiratory symptoms hospitalized between May 2019 and Aug 2019. Children with positive adenovirus test by immunofluorescence assay or PCR in respiratory tract specimens and radiographic findings of pneumonia were included. Children with any of the following factors were excluded: newborns; infection of HIV; leukemia; known or suspected active tuberculosis; receiving immunosuppressive agents; immunodeficiency; chemotherapy; and chronic conditions (malnutrition, congenital heart disease; chronic lung disease).

Classification of pneumonia severity

Classification of pneumonia severity was performed by the criteria of the community-acquired pneumonia guideline in China [7]. Based on the clinical symptoms and chest imaging findings, patients were divided into severe pneumonia group and mild pneumonia group. Severe cases were identified in the presence of at least one of the following signs: disturbance of consciousness, significant tachypnea (respiratory rate > 70 breaths per minute in infants and > 50 breaths per minute in older children), cyanosis, dyspnea, oxygen saturation < 92%, extrapulmonary complication, dehydration, refusal to eat, and severe chest imaging findings (pneumothorax, pleural effusion, pulmonary atelectasis, or multilobe infiltrates).

Data collection and management

The clinical variables were measured every day during hospitalization. Blood draws were done during hospitalization as required for guiding management decisions. Demographic information (age and sex), signs and symptoms (temperature, blood pressure, pulse and respiratory rate, cough, tachypnea, cyanosis, etc.), laboratory results (hematology, organ function, pathogen tests, etc.), chest image results (chest x-ray and/or computed tomography), bronchoscopy results, treatment (oxygen supply, endotracheal intubation, antimicrobial, etc.) and outcome (survival, death, recovery or discharged against medical advice) were recorded. Co-infection of mycoplasma pneumoniae (MP) was defined as positive PCR test of mycoplasma pneumoniae DNA in respiratory tract specimens (oropharyngeal swab or bronchoalveolar lavage fluid) during hospitalization. Co-infection of influenza virus was defined as
positive antigen or PCR test of influenza virus in respiratory tract specimens (nasopharyngeal swab or bronchoalveolar lavage fluid) during hospitalization.

**Sample management and virus detection**

All samples were transported to the laboratory within 4 hours. Respiratory tract samples were tested for adenovirus by D3® UltraTM DFA Respiratory Virus Screening and ID Kit (Diagnostic Hybrids, Inc. USA) or Adenovirus DNA Detection Kit (Shenzhen Puruikang Biotech Co., Ltd). Serum samples were stored at -80°C until adenovirus PCR analysis. Quantification of adenovirus in serum samples was performed on a commercial fluorescence quantitative PCR kit (Daan Gene, Cat. Guangzhou, China) following the protocol of the manufacturer. The limit of detection (LOD) was 500 copies/mL.

**Statistical analysis**

We did a univariate correlation analysis of demographic and laboratory variables to determine the statistical significance of the pairwise associations between the severe and mild pneumonia group. Mann-Whitney test and chi-square test were used for quantitative and qualitative variables, respectively. We further did binary logistic regression analysis to identify independent demographic and laboratory risk factors for severe pneumonia.

Log10-transformed concentrations of serum viral load were used as independent variables in analysis. Serum viral load below the LOD were assigned a viral load of 1 copy/mL (0 log10 copies/mL). Continuous variables were summarized as mean (standard deviation, SD) when they were normally distributed and as median (interquartile range, IQR) if they had a skewed distribution. Sex, age, highest white blood cell (WBC) count during hospitalization, mycoplasma pneumoniae co-infection, influenza virus co-infection, and highest serum viral load in the disease course were applied as independent variables. Data analysis was performed by SPSS 26.0 software. All $P$-values were two-tailed, and $P < 0.05$ was considered to indicate statistical significance.

**Results**

Between May 1, 2019, and Aug 31, 2019, 111 immunocompetent children with adenovirus pneumonia (60 severe, 51 mild) were admitted in hospital and all included (Table 1). The median age was 40 months (IQR 22–64) and 64 patients were male. Bronchoscopy was performed in 47 severe cases and 7 mild cases, where plastic bronchitis was found in 12 severe cases. Five patients were admitted to intensive care unit (ICU) and two of them were endotracheal intubated. None of the patients received anti-adenovirus treatment. All the patients were discharged with recovery or improvement.
We identified demographics and laboratory tests significantly associated with severe adenovirus pneumonia. The median age was 35 months (IQR 21–50) for severe cases and 48 months (IQR 24–72) for mild cases. Male patients accounted for 53% of severe cases and 63% of mild cases. In severe cases, viremia was more common and blood viral loads were generally higher. The highest blood load of adenovirus was observed in a 2-year-old boy with severe pneumonia, plastic bronchitis, pneumothorax, and fungal infection. He was endotracheal intubated. Adenovirus PCR assay in blood was performed on the 26th, 29th, and 39th day of disease course (18th, 21st, and 31st day of hospitalization). The blood viral load result was 6.78 log10 copies/mL, 6.38 log10 copies/mL, and negative respectively. Reduction in viral load paralleled his clinical recovery, which was also seen in the other 6 patients whose blood viral loads were continuously monitored. Among the 12 patients with plastic bronchitis, eight developed viremia. Among the five patients admitted to ICU, three developed viremia. The two endotracheal intubated patients had the highest and 3rd highest blood viral loads, 6.78 log10 copies/mL and 6.09 log10 copies/mL, of all the blood viral load results in this study.
Chi-square test and Mann-Whitney test showed there was a significant difference between severe and mild adenovirus pneumonia in WBC count (12.86 × 10^9/L vs 10.30 × 10^9/L; P = 0.034), co-infection of mycoplasma pneumoniae (70% vs 35%; P< 0.001), presence of viremia (50% vs 24%; P = 0.004) and blood viral load (1.385 log10 copies/mL vs 0 log10 copies/mL; P = 0.001).

We did a binary logistic regression analysis including the following predictors: WBC count, co-infection of mycoplasma pneumoniae and blood viral load (Table 2). In binary logistic regression analysis, leukocytosis (OR = 1.1; 95% CI: 1.0 to 1.2; P = 0.033), co-infection of mycoplasma pneumoniae (OR = 5.0; 95% CI: 2.1 to 12.3; P< 0.001), and high blood viral load (OR = 1.5; 95% CI: 1.2 to 2.0; P = 0.001) were risk factors for severe adenovirus pneumonia.

| Table 2 | Risk Factors for Severe Adenovirus Pneumonia in Immunocompetent Children |
|---------|--------------------------------------------------------------------------------|
|         | Severe Group (n = 60) | Mild Group (n = 51) | P value | Odds Ratio (95% CI) |
| WBC count (10^9/L), range | 12.86 (8.10-16.77) | 10.30 (7.10-14.14) | 0.033 | 1.1 (1.0-1.2) |
| MP co-infection, n % | 42 (70%) | 18 (35%) | < 0.001 | 5.0 (2.1–12.3) |
| Blood viral load (log10 copies/mL), range | 1.385 (0-4.255) | 0 (0–0) | 0.001 | 1.5 (1.2-2.0) |

**Discussion**

From May to August in 2019, an outbreak of adenovirus infection occurred in China. As a common and severe complication of adenovirus infection, adenovirus pneumonia gained our attention. Since severity of adenovirus pneumonia in children varied, we explored useful tools to predict the severity of this disease and guide management decisions. A previous study of immunocompetent children showed adenovirus load in respiratory tract secretions were predictors for disease severity of adenovirus pneumonia [8]. Other previous studies of immunocompromised children have demonstrated an association between adenovirus viremia and disease severity [3–5]. It was not clear whether adenovirus load in blood can predict adenovirus pneumonia severity in immunocompetent children.

In a study involving 4319 children with respiratory tract infection and 361 controls, 16.4% of the 61 available plasma samples were positive for adenovirus DNA and they were all from patients [9]. There was no comparison between severe patients and mild patients. In our study, 50% of severe cases and 24% of mild cases developed viremia. Viremia was significantly more common in severe cases. When serum viral load below the LOD were assigned a viral load of 1 copy/mL, serum viral load in severe cases was also significantly higher than that in mild cases, suggesting a positive correlation between serum viral load and disease severity. Binary logistic regression analysis confirmed the value of serum viral load to predict adenovirus pneumonia severity.
In another study of 196 immunocompetent children with adenovirus respiratory tract infection, adenovirus was detected in blood in 33% of patients and there was no difference in ICU admission between viremia and non-viremia groups [10]. In our study, five patients were admitted into ICU and three of them developed viremia. The two endotracheal intubated patients had the highest and 3rd highest blood viral loads. Though our ICU patient result was not comparable with the previous study for small sample size, it suggested an association between blood viral load and disease severity in ICU patients.

In a case series of adenovirus viremia among previously healthy children, high level viremia was detected in an adenovirus culture-positive 6-month-old girl with pneumonia, conjunctivitis and hepatitis. Subsequent reduction in viral load paralleled her clinical recovery [11]. Another study of 15 immunocompetent adults with adenovirus pneumonia also found that the clinical manifestation recovered gradually with a downward trend in viral load in blood samples [12]. In our study, seven patients recovered with reduction in blood viral load, consistent with the previous study.

Our study also found other risk factors for severe adenovirus pneumonia. Previous studies of adenovirus pneumonia suggested male sex, young age, leukocytosis, and elevated C-reactive protein (CRP) were associated with severe pneumonia [13–15]. In our study, male patients accounted for 63% of mild cases but only 53% of severe cases, which was different from the previous study. The median age was 35 months in severe cases and 48 months in mild cases. The median level of CRP was 33.64 mg/L in severe cases and 26.20 mg/L in mild cases. Though severe cases tended to be younger and have higher CRP, there was no significant difference in univariate analysis. Only leukocytosis was significantly associated with severe adenovirus pneumonia, consistent with previous studies.

MP had been proposed as a cofactor in severe respiratory infections since 1995 [16]. A recent case-control study in China confirmed that children with MP and adenovirus co-infection was relatively more serious than children with single MP infection [17]. In our study, MP co-infection was commonly found in 70% of severe cases and 35% of mild cases. Logistic regression analysis suggested it was significantly related to severe adenovirus pneumonia, consistent with previous studies.

Conclusions
Leukocytosis, co-infection of mycoplasma pneumoniae, and high blood viral load are risk factors for severe adenovirus pneumonia in immunocompetent children. Blood viral load predicts pneumonia severity.

Abbreviations
CI: Confidence interval; CRP: C-reactive protein; ICU: Intensive care unit; IQR: Interquartile range; LOD: Limit of detection; MP: Mycoplasma pneumoniae; OR: Odds ratio; PCR: Polymerase chain reaction; SD: Standard deviation; WBC: White blood cell
Declarations

Acknowledgments

Not applicable.

Authors’ contributions

RMZ acquired clinical data, made contributions to the analysis and interpretation of data, and drafted the manuscript with the help of HMW and SFT. JKD designed the study. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed in strict accordance with the human subject protection guidance of Ministry of Science and Technology of China, and the study protocol was approved by the Ethical Review Committee of Shenzhen Children's Hospital with judgment's reference number 201907903. Written consent was obtained from the guardians of all participants before data collection.

Consent for publication

Not applicable.

Competing interests

The authors have no potential conflicts of interest.

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