Adenovirus Viremia Predicts Adenovirus Pneumonia Severity in Immunocompetent Children

Ruimu Zhang
Shenzhen Children's Hospital
Hongmei Wang
Shenzhen Children's Hospital
Shufeng Tian
Shenzhen Children's Hospital
Jikui Deng (szsetyydeng@sina.com)
Shenzhen Children's Hospital https://orcid.org/0000-0002-0040-1491

Research article

Keywords: Adenovirus, Viremia, Pneumonia, Immunocompetent children

Posted Date: November 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-66081/v2

Version of Record: A version of this preprint was published on February 25th, 2021. See the published version at https://doi.org/10.1186/s12879-021-05903-4.

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 including chest x-ray and computed tomography (CT), bronchoscopy results, treatment (oxygen supply, endotracheal intubation, antimicrobial, etc.) and outcome (survival, death, recovery or discharged against medical advice) were recorded. Mycoplasma pneumoniae (MP) co-infection was defined as positive PCR test of mycoplasma pneumoniae DNA in oropharyngeal swab or bronchoalveolar lavage fluid (BALF) during hospitalization. Viral co-infection was defined as positive antigen or PCR test of other viruses except adenovirus in nasopharyngeal swab or BALF during hospitalization. Bacterial co-infection was defined as positive culture of a single type of bacteria in blood or respiratory tract specimens (sputum, endotracheal aspirate, or BALF) during hospitalization. Fungal co-infection was defined as positive test of

fungi (antigen, antibody, or culture) in blood or respiratory tract specimens with symptoms and chest imaging findings suggesting fungal infection.

Sample management and adenovirus 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℃ 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.

Table 1 Characteristics of Immunocompetent Children with Adenovirus Pneumonia According to Disease Severity

Characteristics	Severe Group (n = 60)	Mild Group (n = 51)	*P* value
Demographics			
Male, n (%)	32 (53%)	32 (63%)	0.317
Age, months (IQR)	35 (21-50)	48 (24-72)	0.052
Clinical features, n (%)			
Bronchoscopy	47 (78%)	7 (14%)	-
Plastic bronchitis	12 (20%)	0	-
ICU admission	5 (8%)	0	-
Laboratory tests			
WBC count, 10^9/L (IQR)	12.86 (8.10-16.77)	10 .30 (7.10-14.14)	0.034
CRP, mg/L (IQR)	33.64 (12.23-68.98)	26.20 (11.70-43.70)	0.091
Co-infection, n (%)	48 (80%)	25 (49%)	0.001
Fungal co-infection, n	2	0	
Bacterial co-infection, n (%)	4 (7%)	4 (8%)	1.000
RSV co-infection, n (%)	4 (7%)	2 (4%)	0.829
Influenza virus co-infection, n (%)	12 (20%)	7 (14%)	0.382
MP co-infection, n (%)	42 (70%)	18 (35%)	< 0.001
Viremia, n (%)	30 (50%)	12 (24%)	0.004
Blood viral load, log10 copies/mL (IQR)	1.385 (0-4.255)	0 (0-0)	0.001

Tests of 7 respiratory viruses (influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza 1, parainfluenza 2, and parainfluenza 3 viruses), MP, and bacterial culture were performed in all the patients. Fungal tests were performed in 2 patients whose chest CT findings suggested fungal infection. One patient had positive BALF culture of aspergillus and the other patient had positive antibody

test of aspergillus. Evidence of viral, bacterial, or fungal co-infection was found in 73 patients (Table 1). None of the patients had positive blood culture of bacteria or fungi.

In mild cases, blood viral load ranged from 0 to 4.54 log10 copies/mL (IQR 0-0). In severe cases, blood viral load ranged from 0 to 6.78 log10 copies/mL (IQR 0-4.255). The highest blood load of adenovirus was observed in a 2-year-old boy with severe pneumonia, plastic bronchitis, pneumothorax, and fungal co-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 (Fig. 1). 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.

We identified demographics and laboratory tests significantly associated with severe adenovirus pneumonia. 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 (80% vs 49%; $P = 0.001$), 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 (IQR)	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 (IQR)	1.385 (0-4.255)	0 (0-0)	0.001	1.5 (1.2-2.0)

As co-infection was common among patients in the study and it may affect the value of viremia in predicting disease severity, we also compared the demographics and laboratory test results among the 38 patients without co-infection using Fisher's exact test and Mann-Whitney test (Table 3). In the mild group, blood viral load ranged from 0 to 4.54 log10 copies/mL (IQR 0-2.638). In the severe group, blood viral load ranged from 0 to 6.13 log10 copies/mL (IQR 0-4.043). Blood viral load was significantly increased in the severe group.

Table 3 Characteristics of Immunocompetent Children with Pneumonia caused by single Adenovirus Infection

Characteristics	Severe Group (n = 12)	Mild Group (n = 26)	*P* value
Demographics			
Male, n (%)	9 (75%)	15 (58%)	0.472
Age (months), IQR	23(15-42)	41 (22-65)	0.113
Laboratory tests			
WBC count (10^9/L), IQR	12.51 (6.07-18.10)	10 .40 (6.75-14.51)	0.683
CRP (mg/L), IQR	25.75 (16.50-51.33)	27.45 (19.95-48.33)	0.962
Viremia, n (%)	8 (67%)	11 (42%)	0.295
Blood viral load (log10 copies/mL), IQR	3.675 (0-4.043)	0 (0-2.638)	0.022

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, 38% of patients developed viremia. Blood 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 were more common in mild cases, which was different from the previous study. Additionally, though severe cases in our study 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 cases. Logistic regression analysis suggested it was significantly related to severe adenovirus pneumonia, consistent with previous studies.

Co-infection of other pathogens may worsen adenovirus pneumonia and make it difficult to evaluate the role of adenovirus viremia in predicting disease severity. Therefore, we did a comparison among the 38 patients with pneumonia caused only by adenovirus. Although WBC count and cases of viremia were not significantly increased in the severe group, possibly due to decreased sample size, blood viral load was still significantly higher. This confirmed the value of blood viral load in predicting severity of adenovirus pneumonia.

This study has limitations due to its retrospective nature and small sample size. First, a qualitative PCR assay was used to detect adenovirus in respiratory tract specimens during the outbreak. The respiratory tract samples had not been saved and the corresponding viral load was unclear. Therefore, the comparison of adenovirus viral loads between respiratory tract samples and blood samples was unable to be made. Second, typing of adenovirus in blood was not performed due to insufficiency of remaining blood samples. Third, a MP PCR assay targeting DNA was used to detect MP, which was difficult to differentiate active infection from carriage. Detection of MP RNA or paired sera antibody test would have provided further evidence of active MP infection. Fourth, the sample size of patients without co-infection was small. The statistical analysis may be more accurate if the sample size was larger.

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

BALF: Bronchoalveolar lavage fluid; CI: Confidence interval; CRP: C-reactive protein; CT: computed tomography; 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.

Funding

Not applicable.

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.

References

1. Munoz FM, Piedra PA, Demmler GJ. Disseminated adenovirus disease in immunocompromised and immunocompetent children. Clin Infect Dis. 1998;27:1194-200.
2. Lankester AC, van Tol MJ, Claas EC, Vossen JM, Kroes AC. Quantification of adenovirus DNA in plasma for management of infection in stem cell graft recipients. Clin Infect Dis. 2002;34:864-7.
3. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. Blood. 2003;102:1114-20.
4. Echavarria M, Forman M, van Tol MJ, Vossen JM, Charache P, Kroes AC. Prediction of severe disseminated adenovirus infection by serum PCR. Lancet. 2001;358:384-5.
5. Leruez-Ville M, Minard V, Lacaille F, et al. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. Clin Infect Dis. 2004;38:45-52.
6. National Health Commission of China. Guideline for diagnosis and treatment of adenovirus pneumonia in children (2019 version). Chin J Clin Infect Dis. 2019;12:161-6.
7. National Health Commission of China. Guideline for diagnosis and treatment of community-acquired pneumonia in Children (2019 version). Chin J Clin Infect Dis. 2019;12:6-13.
8. Xie L, Zhang B, Zhou J, et al. Human adenovirus load in respiratory tract secretions are predictors for disease severity in children with human adenovirus pneumonia. Virol J. 2018;15:123.
9. Schjelderup NH, Nordbo SA, Krokstad S, Dollner H, Christensen A. Human adenovirus in nasopharyngeal and blood samples from children with and without respiratory tract infections. J Clin Virol. 2019;111:19-23.
10. Song E, Wang H, Leber A, Jaggi P. Human Adenovirus (HAdV) Viremia in Immunocompetent Children with HAdV Infection in Respiratory Specimens: Does Viremia Predict Severity of Illness?. Open Forum Infect Dis. 2017;4 (Suppl 1):S358.

11. Shike H, Shimizu C, Kanegaye J, Foley JL, Burns JC. Quantitation of adenovirus genome during acute infection in normal children. Pediatr Infect Dis J. 2005;24:29-33.
12. Gu L, Qu J, Sun B, Yu X, Li H, Cao B. Sustained Viremia and High Viral Load in Respiratory Tract Secretions Are Predictors for Death in Immunocompetent Adults with Adenovirus Pneumonia. Plos One. 2016;11:e160777.
13. Chuang Y, Chiu CH, Wong KS, et al. Severe adenovirus infection in children. J Microbiol Immunol Infect. 2003;36:37-40.
14. Miao H, Rong L, Zhou F. Risk factors for poor prognosis in children with severe adenovirus pneumonia. Chin J Contemp Pediatr. 2017;19:159-62.
15. Mingyue L, Linying G, Dong Q, et al. Clinical analysis of children in hospital with adenovirus pneumonia in Beijing from 2015 to 2016. Chin J Exp Clin Virol. 2018;32:62-5.
16. Cimolai N, Wensley D, Seear M, Thomas ET. Mycoplasma pneumoniae as a cofactor in severe respiratory infections. Clin Infect Dis. 1995;21:1182-5.
17. Gao J, Xu L, Xu B, Xie Z, Shen K. Human adenovirus Coinfection aggravates the severity of Mycoplasma pneumoniae pneumonia in children. BMC Infect Dis. 2020;20:420

Figures

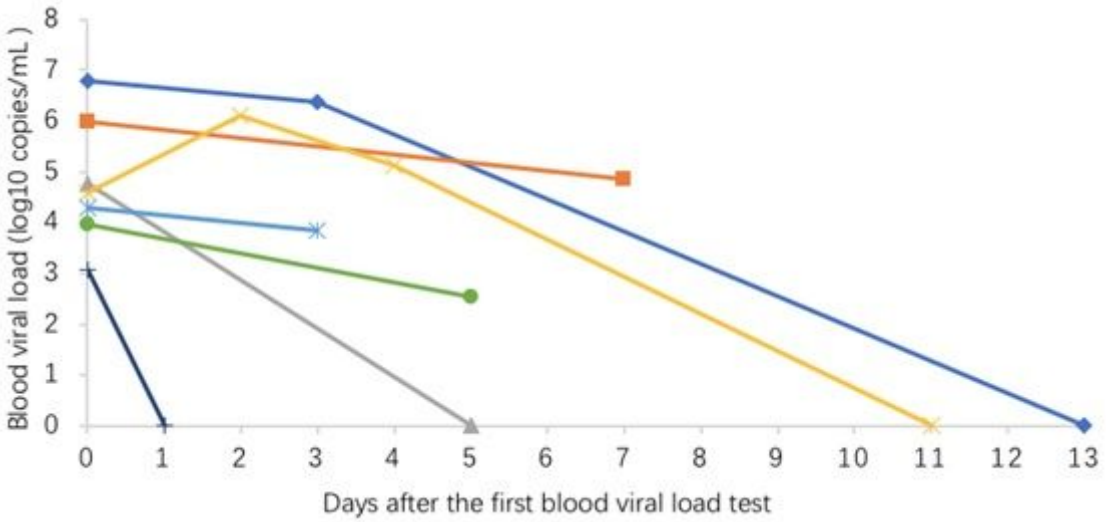

Figure 1

Blood viral load of 7 patients with adenovirus pneumonia whose viremia status was continuously monitored. The viral load below the limit of detection was assigned 0 log10 copies/mL.

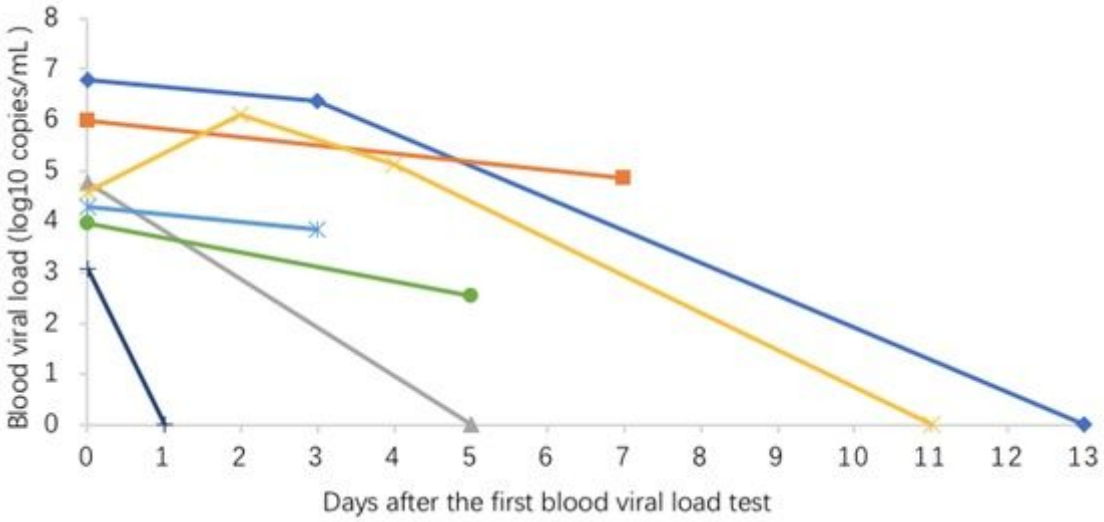

Figure 1

Blood viral load of 7 patients with adenovirus pneumonia whose viremia status was continuously monitored. The viral load below the limit of detection was assigned 0 log10 copies/mL.