

Risk Factors for Severe Adenovirus Infection in Children during an Outbreak in Singapore

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Abstract

Background: Human adenoviruses (HAdVs) can cause a variety of human illnesses, with associated temporal and geographic changes in disease incidence. We report the emergence of an outbreak of HAdV infections in Singapore, presumably caused by a change of the predominating type to HAdV-7. We examined the clinical features of children admitted with HAdV infection to 1 institution and the risk factors for severe infection. **Materials and Methods:** This is a retrospective case-control study of all HAdV-infected children admitted during weeks 1 to 19 in 2013, as identified from laboratory records. A descriptive retrospective analysis of epidemiology, clinical data and the outcome of these children was also performed. Patients with severe infections were defined as cases, those with non-severe infections as controls, and the 2 groups were compared to find possible independent risk factors. **Results:** Eighty-five patients with HAdV infection were studied, including 11 (12.9%) cases and 74 (87.1%) controls. Binary logistic regression showed that cases were more likely to be <2 years old (adjusted OR 10.6, 95% CI, 1.8 to 63.2) and to have significant comorbidities (adjusted OR 19.9, 95% CI, 3.4 to 116.1) compared to controls. The predominant type in 2013 was HAdV-7, which differed from 2011 and 2012, when HAdV-3 was more common. There was a trend towards pneumonia being more common in patients infected with HAdV-7 than in patients infected with other types, although this did not reach statistical significance (OR 2.8, 95% CI, 0.9 to 8.7). **Conclusion:** The emergence of HAdV-7 in a population where other HAdV types had circulated previously may have caused the outbreak in Singapore, and this was associated with more serious infections in children. Young age (<2 years) and significant comorbidities were associated with more severe HAdV infection.

Ann Acad Med Singapore 2015;44:50-9

Key words: Comorbidity, Complications, Epidemiology, Immunocompromised, Paediatric

Introduction

Human adenoviruses (HAdVs) are well known pathogens that cause a variety of human illnesses. They are non-enveloped, linear double-stranded deoxyribonucleic acid (DNA) viruses. More than 50 distinct serotypes have been identified since the early 1950s. The wide spectrum of symptoms includes upper respiratory tract illness, pneumonia, conjunctivitis, cystitis and gastroenteritis.^{1,2} Characteristic illnesses associated with HAdVs are pharyngoconjunctival fever, epidemic keratoconjunctivitis and acute respiratory illnesses in military recruits. Immunocompromised persons are known to be affected by more severe disease.^{3,4} The

virus spreads rapidly in crowded environments, typically causing epidemics in military recruits and hospitalised or institutionalised civilians.^{5,6} In the paediatric population, particularly in children with underlying respiratory disease, the illness can be severe and life-threatening.⁷ Due to a large proportion of infections being subclinical and due to a lack of surveillance for HAdV infections in many countries, most community outbreaks remain unreported. A community outbreak of adenovirus was reported in Taiwan in 2011, using a vigilant surveillance system comprising 12 contract virological laboratories across the country.⁸

We noticed an apparent increase in the incidence of HAdV

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infections at our institution, a tertiary-care paediatric hospital in Singapore, KK Women's and Children's Hospital (KKH), during the first half of 2013. This was based on an increase of HAdV-positive specimens identified via microbiology laboratory records, and an apparent increase of patients requiring hospital admission, including patients with severe disease. In the present article, we report some of the characteristics of this outbreak and provide an analysis of the clinical features and risk factors associated with severe HAdV infections in children.

Methods

We conducted a retrospective case-control study combined with a descriptive analysis of epidemiology, clinical data and outcome among children with HAdV infections admitted to our tertiary-care paediatric hospital in Singapore. The HAdV-positive patients were identified from microbiology laboratory records over the period from 1 January 2013 to 13 May 2013 (weeks 1 to 19 in 2013). Children between the ages of 1 month and 17 years were included in the study. Factors such as demographics, clinical presentation, severity of illness, complications and management were recorded. For the purpose of the case-control study, HAdV-positive subjects, whose severity of illness required admission to the high dependency (HD) unit or the children's intensive care unit (CICU) were defined as 'cases'. The remaining HAdV-positive subjects, who did not require HD or CICU admission, were classified as 'controls'. Admission to the HD unit or the CICU was based on clinical judgment by the attending physicians, in combination with respiratory, circulatory or mental state parameters that were indicative of severe illness. Respiratory distress, including the requirement for supported ventilation, was the commonest reason for escalation of care to the HD unit or CICU. Others included a requirement for circulatory support or close monitoring for changes in mental status.

Based on clinical presentation, testing for HAdV was done on relevant samples using the following methods: (a) specific (genus-wide) HAdV real-time polymerase chain reaction (PCR), based on an assay by Heim et al;⁹ (b) respiratory virus direct immunofluorescence (IF) test (D3 Double Duet™ DFA Respiratory Virus Screening & ID Kit, Diagnostic Hybrids, Athens, OH, USA); and (c) respiratory virus multiplex PCR (Seeplex RV15 ACE Detection Kit, Seegene, Seoul, Korea). Positive samples were forwarded to the Singapore National Public Health Laboratory (NPHL) for further analysis, including virus culture (when feasible) and genotyping. HAdV genotyping was performed using a method developed by Lu and Erdman,¹⁰ by sequence comparison of a ~750 bp segment containing hexon gene hypervariable regions 1-6 (HVR 1-6). Data on HAdV types were then compared with other

clinical and epidemiological data.

The virus panel in the routine IF test consisted of influenza A and B viruses, HAdV, parainfluenza 1, 2 and 3 viruses, respiratory syncytial virus and metapneumovirus, and the multiplex PCR panel contained, in addition, parainfluenza 4 virus, human coronaviruses 229E, NL63 and OC43, enterovirus and bocavirus. It is common hospital practice at KKH to request the IF panel for most respiratory tract infections in children, while the multiplex PCR panel is usually requested for more difficult or severe cases. In addition, routine bacteriology tests are requested based on clinical indication.

Statistical analysis was performed using the SPSS statistical software programme, version 19 (IBM Corporation, Armonk, NY, USA). The chi-square tests and Fisher's exact tests were used for categorical data, and the t-test and Mann-Whitney U test for continuous data. Binary logistic regression analysis was used to examine risk factors that were significant in the univariate analysis. Forward stepwise regression was used for identifying the optimum variables for the model. This study was approved by the Singhealth institutional review board and approval was obtained for a waiver of patient consent.

Results

Epidemiology

From week 1 to 19 of 2013, 85 patients admitted to KKH had positive test results for HAdV. Eleven patients (12.9%) had severe infections, required admission to HD or CICU, and were defined as cases, and 74 children (87.1%) had non-severe infections, did not require admission to HD or CICU and were defined as controls.

Among the 85 HAdV-positive patients, 64 (75.3%) had HAdV detected by IF, and 21 (24.7%) had HAdV detected by PCR in nasopharyngeal swabs. Twenty of the 21 PCR-positive patients had initially been negative by IF testing and were subsequently tested by PCR, in view of a strong clinical suspicion of HAdV infection. Three out of the 85 patients had HAdV detected from additional sites. One patient (designated Patient A) had positive PCR results from blood, stool and a colon biopsy (post bone marrow transplant with adenovirus infection causing colitis), 1 patient had a positive HAdV PCR result from urine, and 1 patient (designated Patient B) with acute respiratory distress syndrome (ARDS) had a positive HAdV PCR result from a bronchoalveolar lavage specimen.

Demographics and Underlying Comorbidities

The median age of our patients was 34 months, with a mean age of 48.6 months (range, 3 to 187 months), and 53 (62.4%) were males. The ethnicities of the patients were

Chinese (42.4%), Malay (41.2%), Indian (12.9%), and others (3.5%). Fourteen patients (16.5%) had significant underlying comorbidities, such as cardiopulmonary diseases that included tracheobronchomalacia and pulmonary hemosiderosis (8.2%), neurological diseases that included methyl-CpG-binding protein 2 (*MECP2*) duplication syndrome and spastic quadriplegic cerebral palsy (4.7%), immunodeficiency, consisting of X-linked chronic granulomatous disease (2.4%), and haemato-oncological diseases, consisting of chronic idiopathic thrombocytopenic purpura (1.2%).

Clinical and Laboratory Characteristics

Clinical characteristics of the HAdV-positive patients are shown in Table 1. All cases were examined by a senior doctor in paediatrics (rank of paediatric registrar and above). The most common symptoms were fever (97.6%), cough (80%) and coryza (76.5%). Conjunctivitis was present in 18 patients (21.2%). The mean duration of fever was 7.4 days (range, 0 to 20 days). Physical examination findings included lung crepitations (12.9%) and rhonchi (11.8%). The most common HAdV-related presentations were upper respiratory tract infection (55.3%), pneumonia (22.4%), gastroenteritis (7.1%) and tonsillitis (7.1%). Severe disease complications included pleural effusion (4.7%), with half of them (2/4, 2.4%) requiring chest tube drainage, acute respiratory distress syndrome (ARDS; 2.4%), disseminated intravascular coagulopathy (DVC; 3.5%), seizures (3.5%) and encephalopathy (2.4%). A total of 54 of 85 (63.5%) patients had at least 1 complication (a list of complications is provided in the section 'Comparison between cases and controls').

Laboratory investigations are shown in Table 2 which includes a comparison between cases and controls. Statistical

Table 1. Clinical Characteristics of HAdV-positive Patients (n = 85)

Characteristics	Number of Patients (%)
Symptoms	
Fever	83 (97.6)
Conjunctivitis	18 (21.2)
Rash	8 (9.4)
Cough	68 (80.0)
Coryza	65 (76.5)
Sore throat	11 (12.9)
Diarrhoea	25 (29.4)
Vomiting	30 (35.3)
Abdominal pain	12 (14.1)
Signs	
Lung crepitations	11 (12.9)
Lung rhonchi	10 (11.8)
Stridor	1 (1.2)
Hepatomegaly	3 (3.5)
Splenomegaly	2 (2.4)
Lymphadenopathy	2 (2.4)

HAdV: Human adenovirus

significance analysis (Mann-Whitney U test) was not done for results that had a skewed distribution due to a wide range of normal values. When clinically indicated, further microbiological testing was done for bacterial and other viral pathogens. Coinfections with other presumptive pathogens were seen in 12 of 85 (14.1%) patients (Table 3). Viral coinfections were detected in 5 patients (5.9%) and included bocavirus, rhinovirus, parainfluenza virus and enterovirus. Six patients (7.1%) had concurrent bacterial infection or colonisation with potential pathogens, and 1 patient had coinfection with fungi.

Table 2. Laboratory Investigations Done in HAdV-positive Patients, with a Comparison between Cases and Controls

Laboratory Results (Median Values) and Number of Patients Tested	All	Cases	Controls	P Value
Haemoglobin, g/dL (range), n = 80	11.6	8.7 (6.8 – 11.4)	11.7 (8.9 – 15.4)	<0.0001
Leukocytes, $\times 10^3$ cells/ μ L (range), n = 80	11.5	5.6 (1.4 – 46.6)	11.5 (3.2 – 32.6)	0.279 (NS)
Neutrophils, $\times 10^3$ cells/ μ L (range), n = 80	6.1	3.3 (0.7 – 21.0)	6.2 (1.0 – 28.7)	0.137 (NS)
Lymphocytes, $\times 10^3$ cells/ μ L (range), n = 80	6.1	1.2 (0.1 – 1.6)	2.8 (0.9 – 12.1)	<0.0001
Platelets, $\times 10^3$ / μ L (range), n = 80	233	132 (29 – 376)	241 (35 – 575)	0.046
Urea, mg/dL (range), n = 58	3.0	4.1 (2.7 – 26.6)	2.8 (1.3 – 6.4)	0.001
Creatinine, μ mol/L (range), n = 58	43	46 (38 – 209)	43 (32 – 67)	0.204 (NS)
Sodium, mmol/L (range), n = 58	136	134 (131 – 140)	137 (131 – 140)	*
ALT, IU/L (range), n = 22	16	41 (10 – 1033)	15 (8 – 76)	*
AST, IU/L (range), n = 22	32	43 (17 – 1374)	28 (15 – 78)	*
CRP, mg/dL (range), n = 48	35.8	45.7 (7.2 – 348.7)	34.8 (0.7 – 185.4)	*

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein; HAdV: Human adenovirus; NS: Not significant.

*Statistical significance analysis (Mann-Whitney U test) was not done for results with skewed distribution due to wide range of normal values.

Table 3. Coinfecting Pathogens in HAdV-positive Patients

Organisms Identified	Number of Patients (%)
Viral	5 (5.9)
Bocavirus	1 (1.2)
Bocavirus, parainfluenza 1 virus	1 (1.2)
Enterovirus	1 (1.2)
Metapneumovirus	1 (1.2)
Rhinovirus	1 (1.2)
Bacteria	6 (7.1)
<i>Stenotrophomonas maltophilia</i> (ETTA)	2 (2.4)
<i>Acinetobacter baumannii</i> (tracheostomy aspirate)	1 (1.2)
<i>Escherichia coli</i> (urine)	1 (1.2)
<i>Moraxella catarrhalis</i> , <i>Klebsiella</i> species (ETTA)	1 (1.2)*
<i>Haemophilus influenzae</i> (ear and eye swab)	1 (1.2)*
<i>K. pneumoniae</i> (blood)	1 (1.2)
Fungi	1 (1.2)
<i>Candida albicans</i> , <i>C. dubliniensis</i> , <i>Aspergillus fumigatus</i> , <i>A. terreus</i> (BAL)	1 (1.2)†

BAL: Bronchoalveolar lavage; ETTA: Endotracheal tube aspirate; HAdV: Human adenovirus

*These coinfecting pathogens were isolated from different sample sites of the same patient.

†This patient with trisomy 21 and pulmonary haemosiderosis had 4 fungi in addition to adenovirus.

HAdV Typing

Adenovirus genotyping was successful in 76 (89.4%) samples (Table 4). Almost all HAdV isolates from KKH were genotyped in 2012 and 2013 but only 15% were genotyped in 2011. The majority of patients (44 out of 76 samples, 57.9%) were found to be infected with HAdV-7. This was in contrast to available paediatric genotyping data from

NPHL obtained in 2011 and 2012, where the predominant type was HAdV-3, and HAdV-7 accounted for none and only 9.7% respectively, of the total HAdV serotypes. A statistical comparison of HAdV-7 versus non-HAdV-7 types between the 2012 and 2013 study period showed a statistically significant shift towards HAdV-7 in 2013 (OR 12.8, 95% CI, 5.2 to 31.5). There was a trend towards pneumonia being more common in patients infected with HAdV-7 (15/44) than in patients infected with other types (5/32), although this did not reach statistical significance (OR 2.8, 95% CI, 0.9 to 8.7).

Management and Outcome

Intravenous hydration was required in 40% of patients in view of poor oral intake. Oxygen supplementation was necessary in 14.1% of patients, and 16.5% of the children also required bronchodilators. One of the 7 cases (designated Patient C) who required assisted ventilation received non-invasive ventilation, as the parents opted for non-escalation of care in view of poor premorbid status, and the patient eventually passed away from the illness. Another patient (designated Patient B) required extracorporeal membrane oxygenation (ECMO) for severe ARDS. She had underlying pulmonary hemosiderosis requiring long-term corticosteroids, trisomy 21, polyarticular juvenile idiopathic arthritis and hypothyroidism, and died from her illness.

Antibiotics were started empirically for clinically suspected pneumonia and given orally in 44.7% and parenterally in 35.3% of the patients. Intravenous cidofovir was given to 3 patients with severe illness, at the discretion of the attending paediatric infectious diseases specialist, at a dose of 5 mg/kg once a week, together with probenecid. However, 2 of these patients had severe disease with ARDS and eventually died. The third patient (Patient A) had colitis secondary to adenovirus infection on day 28 after bone marrow transplant. Cidofovir was given for 2 doses weekly and then discontinued after the patient's symptoms had

Table 4. Adenovirus Typing Results

Type	Cases (n = 11, %)	Controls (n = 65, %)	Odds Ratio (95% CI)	2013* (n = 76, %)	2012 (n = 72, %)	2011 (n = 47, %)†
1	0	1 (1.5)		1 (1.3)	9 (12.5)	10 (21.3)
2	0	2 (3.1)		2 (2.6)	5 (6.9)	6 (12.7)
3	1 (9.1)	6 (9.2)		7 (9.2)	24 (33.3)	25 (53.1)
4	2 (18.2)	19 (29.2)		21 (27.6)	16 (22.2)	2 (4.2)
5	0	1 (1.5)		1 (1.3)	5 (6.9)	0
7	8 (72.7)	36 (55.4)	2.1 (0.5 – 8.8)	44 (57.9)	7 (9.7)	0
8	0	0		0	2 (2.7)	4 (8.5)
Others (15, 19, 41)	0	0		0	4 (5.5)	0

*The 2013 HAdV typing data is for the study period Jan-May 2013.

†Only 15% of HAdV isolates from KKH were typed in 2011, whereas almost all isolates were typed in 2012 and 2013.

improved. The mortality rate for the entire cohort of patients with HAdV infection was 3.5% (3 deaths). This included the 2 patients previously mentioned (Patient B and Patient C) and another one who had spastic quadriplegic cerebral palsy and was dependent in all activities of daily living.

On discharge, 1 patient who was previously well, had neurological sequelae of oropharyngeal dysphagia requiring nasogastric tube feeding. One patient had respiratory sequelae and was discharged on continuous positive airway pressure (CPAP) ventilation with oxygen. This patient had a history of *MECP2* duplication syndrome with global developmental delay but had been able to breathe in ambient air before his admission with HAdV infection.

Comparison between Cases and Controls

Table 5 shows an analysis to compare demographics, disease presentation, complications and treatment of cases ($n = 11$) with controls ($n = 74$). There was no statistical difference between the gender of the cases and controls. Although our study population included a higher proportion of patients of Malay ethnicity compared to the general population of Singapore (13.3% in 2013), there was no statistical difference in ethnicity between of the cases and controls. Cases were more likely to be <2 years old compared to controls (Pearson chi-square $P = 0.003$, OR 7.2, 95% CI, 1.7 to 29.9). Significant comorbidities were more likely to be seen in cases than controls (OR 16.8, 95% CI, 3.9 to 71.7). Binary logistic regression (using the variables significant in univariate analysis) confirmed the above findings, that cases were more likely to be <2 years (adjusted OR 10.6, 95% CI, 1.8 to 63.2) and to have significant comorbidities (adjusted OR 19.9, 95% CI, 3.4 to 116.1) compared to controls. Patients presenting with pneumonia were more likely to be complicated cases compared to those presenting with upper respiratory tract infection (URTI), bronchiolitis, bronchitis or tonsillitis ($P < 0.0001$).

Renal impairment, significantly elevated transaminases and haematological changes including lymphopenia, thrombocytopenia and anaemia were more likely to be present in cases rather than controls. The other obvious finding expected from the classification applied, was that, complications such as DIVC, pleural effusion requiring drainage, ARDS requiring invasive ventilatory support or ECMO, and hypotension requiring inotropic support, occurred significantly more frequently in cases compared to controls. Viral coinfections were more likely to be seen in cases than in controls (3/11 in cases and 2/74 in controls; $P = 0.001$). Similarly, concurrent bacterial infection or colonisation with potential pathogens were also more likely to be present in cases (4/11 in cases and 2/74 in controls; $P < 0.0001$).

Among those patients for whom HAdV genotyping was available, type 7 was found more commonly in cases (8/11, 72.7%) compared to controls (36/65, 55.4%), but the difference was not statistically significant (OR 2.1, 95% CI, 0.5 to 8.8). Of the 3 patients who died, 2 were infected with HAdV-7 and 1 with HAdV-4.

Discussion

This article describes an apparent increase in the numbers of admissions of patients with HAdV infections to a tertiary-care children's hospital in Singapore. Figure 1 depicts the trend in the numbers of HAdV positive paediatric samples in our institution during the preceding 10 years, as determined by IF testing. Prior to 2013, on average, the numbers of HAdV-positive samples per month were less than 5 to 10 during non-peak periods and up to about 15 to 25 during peak periods. The different peak and non-peak periods presumably reflect fluctuations of virus activity in the community over the 10-year period. The study period in the first half of the year 2013 stands out, with peaks of 25 positive samples in each, March and April, and 42 in May. The total numbers of samples submitted for IF testing (Fig. 1) increased over the years and a direct comparison between 2013 and earlier years from 2003 to 2009 is difficult due to smaller numbers of samples submitted for testing in those years. Nevertheless, the number of samples tested for HAdV plateaued from 2010 onwards and remained nearly unchanged. The hospital's HAdV sampling and testing practices by IF also remained unchanged during the 10-year period. This suggests that there was a distinct change in HAdV epidemiology in 2013, perhaps similar to a smaller peak period in 2011, that led to an increase of patients with HAdV infection seen at our hospital (Fig. 1).

During the study period, the clinical course of the admitted patients showed a substantial proportion of clinical complications (63.5%) and mortality (3.5%). A recent study from China identified HAdV as a cause in 9.6% (13 out of 136 respiratory pathogens identified in 105 patients) of acute respiratory infections (ARI), and the majority occurred in children under the age of 6 years (11/13).¹¹ This is in keeping with a previous study that showed HAdV to be involved in 5% to 10% of ARIs in children, and to be commonly occurring together with other viruses.¹² Coinfections with viruses occurred in 5.9%, and concurrent bacterial infection or colonisation in 7.1% of our patients.

In our study, HAdV illness tended to be more severe in children less than 2 years of age. Previous reports looking at risk factors for severe HAdV infections do not clearly show young age as a risk factor for severe illness.⁸ On the other hand, it is well documented that children with underlying neurological, metabolic or respiratory conditions

Table 5. Univariate Comparison of Cases and Controls

Characteristic	Cases (n = 11)	Controls (n = 74)	Odds Ratio (95% CI)
Age (years)			
Mean	2.6	4.3	
Median	1.4	3.2	
Range	8 months to 13.7 years	3 months to 15.6 years	
Age group, n			
<2 years	8	20	7.2 (1.7 – 29.9)
≥2 years	3	54	
Male, n (%)	6 (54.5)	47 (63.5)	1.1 (0.3 – 4.3)
Ethnicity, n (%)			
Chinese	4 (36.4)	32 (43.2)	
Malay	4 (36.4)	31 (41.9)	
Indian	3 (27.3)	8 (10.8)	
Others	0	3 (4.1)	
Significant comorbidities			
Immunodeficiency	1	1	
Neurological	4	0	
Cardiopulmonary	2	5	
Haematological	0	1	
Total, n (%)	7 (63.6)	7 (9.5)	16.8 (3.9 – 71.7)
Disease presentation, n (%)			
Pneumonia	9 (81.8)	10 (13.5)	
URTI	0	47 (63.5)	
Gastroenteritis	0	6 (8.1)	
Bronchiolitis/bronchitis	0	3 (4.1)	
Tonsillitis	0	6 (8.1)	
Haemorrhagic cystitis	0	1 (1.4)	
Encephalopathy	0	1 (1.4)	
Pneumonia with Encephalopathy	1 (9.1)	0	
Colitis	1 (9.1)	0	
Complications, n (%)			
Pleural effusion (conservative treatment)	1 (9.0)	1 (1.4)	7.3 (0.4 – 126.1)
Pleural effusion (requiring drainage)	2 (18.2)	0	
ARDS	2 (18.2)	0	
DIVC	3 (27.3)	0	
Encephalopathy	1 (9.0)	1 (1.4)	7.3 (0.4 – 126.1)
Seizures	2 (18.2)	1 (1.4)	16.2 (1.3 – 197.3)
Renal impairment	3	0	
Elevated transaminase ALT >2x normal	5 (n = 7)	2 (n = 15)	16.3 (1.8 – 148.8)
Elevated transaminase ALT >2x normal	5 (n = 7)	2 (n = 15)	16.3 (1.8 – 148.8)

ALT: Alanine aminotransferase; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; DIVC: Disseminated intravascular coagulopathy; NS: Not significant; URTI: Upper respiratory tract infection

*Haemoglobin <10 g/dL

†Leukocyte count <5000 cells/μL

‡Neutrophil count <1000 cells/μL

§Lymphocyte count <1000 cells/μL

||Platelet count <150,000/μL

Table 5. Univariate Comparison of Cases and Controls (Con't)

Characteristic	Cases (n = 11)	Controls (n = 74)	Odds Ratio (95% CI)
Anemia*	8 (n = 11)	4 (n = 69)	43.3 (8.2 – 229.6)
Leukopenia†	7 (n = 11)	2 (n = 69)	19.1 (3.0 – 123.9)
Neutropenia‡	1 (n = 11)	1 (n = 69)	6.8 (0.4 – 117.6)
Lymphopenia§	4 (n = 11)	4 (n = 69)	9.3 (1.9 – 45.5)
Thrombocytopenia	5 (n = 11)	5 (n = 69)	10.7 (2.4 – 47.6)
Treatment given, n (%)			
Oxygen	9 (81.2)	3 (4.1)	106.5 (15.6 – 725.6)
Bronchodilators	9 (81.2)	5 (6.8)	62.1 (10.5 – 368.6)
Intravenous cidofovir	3 (27.3)	0	

ALT: Alanine aminotransferase; ARDS: Acute respiratory distress syndrome; AST: Aspartate aminotransferase; DIVC: Disseminated intravascular coagulopathy; NS: Not significant; URTI: Upper respiratory tract infection

*Haemoglobin <10 g/dL

†Leukocyte count <5000 cells/ μ L

‡Neutrophil count <1000 cells/ μ L

§Lymphocyte count <1000 cells/ μ L

||Platelet count <150,000/ μ L

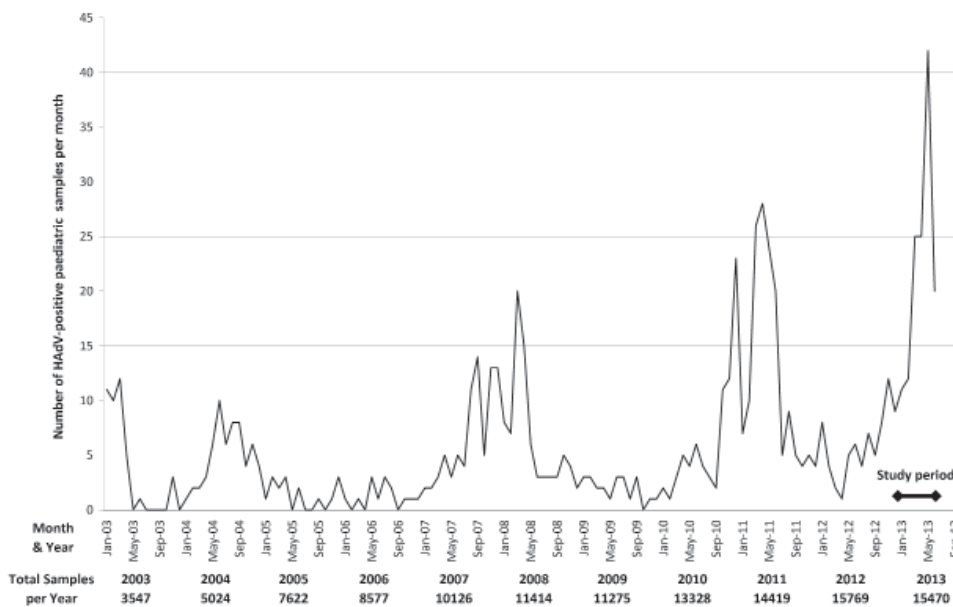


Fig.1. Trend of HAdV-positive paediatric specimens over 10 years, between January 2003 and June 2013. The figure shows the absolute numbers of HAdV-positive specimens per month, as determined by the respiratory virus direct immunofluorescence test. The period of the present report (January 2003 to May 2013) is indicated. The total numbers of samples submitted each year for IF testing is also shown along the X axis for reference.

are more likely to develop more severe illness.¹³⁻¹⁶ In addition to neurological or pulmonary disease, children with other comorbidities, including immunodeficiency and cardiopulmonary disease, were also more likely to have severe disease in our study. The complications of pleural effusion and haematological cytopenias found in severe infections (cases) were comparable to those found in a similar study in Taiwan in 2011.⁸ We further report 2 severe cases with ARDS, one of these requiring ECMO, and both patients eventually passed away despite cidofovir treatment. A third death occurred in a child with

an underlying neurological condition (Patient C). Severe illness was, however, also observed in 4 previously healthy children with no relevant pre-existing comorbidities.

The role of HAdV in the aetiology of severe acute respiratory illnesses in immunocompromised patients is well documented.¹⁷ Depending on the immunosuppressive regimen, rates of HAdV infection of 2% to 21% with a mortality rate ranging from 10% to 60% have been described.¹⁸ Most HAdV infections, however, are mild, self-limiting illnesses. Different serotypes are associated with different clinical manifestations by virtue of variable tissue

tropism. *HAdV-3* has been identified in several outbreaks across different continents over the years.¹⁹ Greater severity of this generally benign *HAdV-3* serotype has been reported in a paediatric long-term facility outbreak in Illinois in 2005, associated with substantial morbidity and mortality in children with underlying respiratory conditions.²⁰ Several adenovirus outbreaks have been reported in Asia. Notable ones have been reported from Taiwan; this includes one between 1999 and 2001, involving serotypes *HAdV-3*, -7 and -4,²¹ another in 2004 to 2005 with *HAdV-3*,²² and more recently, an outbreak in 2011, with types 3 and 7 causing severe respiratory infections with respiratory failure in children.²³ Concurrent circulation of *HAdV-3* and *HAdV-7* during outbreaks has been reported in other populations.^{8,24}

HAdV-7 has previously been reported to be associated with severe clinical illness and morbidity.^{13,25,26} In China, *HAdV-7* contributed to an outbreak of pneumonia in infants in 2011²⁵ and was also reported to have a higher case fatality rate compared to *HAdV-3*.¹⁴ In a case cluster in California, *HAdV-7b* was involved in severe respiratory illness in children and associated with a high number of deaths; 4 out of 6 patients (66.6%) with *HAdV-7b* died of the infection.²⁷ Another outbreak of *HAdV-7* infection, causing life-threatening disease, was reported in an institution for disabled children.¹³ A high case-fatality rate with *HAdV-7* was also reported from Taiwan in 2010 to 2011. Among severe cases requiring intensive care, the mortality rate for serotype 7 was 32%, compared to 14% for non-serotype 7 infections.²³ Although different *HAdV-7* subtypes have been reported in various parts of the world to be associated with outbreaks and severe clinical infections,²⁸⁻³¹ a serotype prevalence and risk factor study in USA in 2004 to 2006 did not show a strong association of *HAdV-7* with severity of infection.¹⁵ Therefore, there seems to be conflicting evidence for the association of *HAdV-7* with severe clinical disease.

Our experience in the paediatric population in Singapore in previous years showed *HAdV-3* to be the predominant serotype among the isolates that were typed (33.3% in 2012 and 53.1% in 2011). *HAdV-7* was not isolated in any of the samples serotyped in 2011. There were less *HAdV-7* among the samples in 2012 (9.7%, 7/72) compared to the high number (51.8%, 44/76) in 2013, thus showing the emergence of a different predominant serotype causing respiratory illness among hospitalised children. Strict infection control measures were employed to prevent nosocomial spread of HAdV in our hospital.

Treatment of HAdV infections has not been well studied; in particular, information from well designed clinical trials is lacking, but from the available information, cidofovir is considered the drug of choice for severe HAdV infections.³² In immunocompromised patients, especially in the scenario after stem cell transplant, where reactivation and horizontal

transmission may cause severe and life threatening illness,³³ cidofovir is the most accepted antiviral therapy.³⁴ Three of our 11 patients (27.3%) with severe HAdV disease, who also had underlying comorbidities, were treated with cidofovir, 2 of whom (including patient B) died of adenovirus-associated ARDS. The decision to treat with cidofovir was at the discretion of the infectious diseases specialist, based on the severity of the illness and presence of underlying comorbidities. The third patient treated with cidofovir (Patient A) had colitis and survived. Of the 8 patients with severe HAdV disease who were not treated with cidofovir, 4 had comorbidities (ex-prematurity, neuroregressive disorder, *MECP2* duplication syndrome and tracheobronchomalacia). Of these, 3 recovered, and the patient with neuroregressive disorder (Patient C) died. In terms of preventative measures, vaccines would be a potentially attractive option, but the only formulation that is currently available is used solely for US military recruits for preventing acute respiratory disease.^{24,35} Currently, there is no HAdV vaccine available for use by the general public.

The main side effect limiting the use of cidofovir is nephrotoxicity. None of our 3 patients treated with intravenous cidofovir showed apparent drug-related side effects. Safety, efficacy and a better side-effect profile with lesser nephrotoxicity has been shown with the use of hexadecyloxypropyl cidofovir (CMX001), an orally bio-available lipid conjugate of cidofovir in a study of immunocompromised HAdV-infected patients.³⁶

Our study has 2 main limitations. First, the decision to admit patients to the HD unit or to the CICU—which determined whether a patient would be designated as a ‘case’ or a ‘control’—was based on a combination of clinical judgement, pathophysiological and laboratory parameters, mostly before HAdV test results were available. Thus, our classification as severe or non-severe, which formed the basis of our risk factor analysis, was not solely based on objective criteria, and this may have unknowingly influenced our analyses. Second, our cohort of 11 severe cases as well as our overall number of patients was relatively small, and this has had a limiting effect on the statistical power of our analyses. For example, it is possible that the comparison of pneumonia cases caused by *HAdV-7* versus the other types would have reached statistical significance if the sample size had been bigger. Nevertheless, both limitations reflect ‘real-life’ conditions encountered during a HAdV outbreak at a single tertiary-care institution, and our observations add to the growing knowledge concerning the epidemiological and clinical features of HAdV infections.

Conclusion

HAdV infections can cause community outbreaks, and severe HAdV infections can be seen especially with *HAdV-*

7. In the present study, severe infection was found more frequently in patients <2 years old and those with underlying comorbidities. A change in serotype from HAdV-3 in 2011 to HAdV-7 in 2013 may have contributed to this outbreak in Singapore that resulted in an increase in HAdV-related paediatric admissions.

Acknowledgements

The authors would like to thank the staff of the Molecular Microbiology Laboratory, KK Women's and Children's Hospital and the staff of National Public Health Laboratory, Singapore, for their help and technical assistance. The authors would also like to thank Dr Lek Ngee from the Department of Paediatrics, KK Women's and Children's Hospital for his contribution in preparing the manuscript.

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