

Adenovirus Type 21 Outbreak among Lung Transplant Patients at a Large Tertiary Care Hospital

Sarah E. Philo^{1,2}, Benjamin D. Anderson^{1,2,3}, Sylvia F. Costa¹, Nancy Henshaw⁴, Sarah S. Lewis¹, John M. Reynolds⁵, Jayanthi Jayakumar⁶, Yvonne C. F. Su⁶,
and Gregory C. Gray^{1,2,3,6}

¹ Division of Infectious Disease, School of Medicine, Duke University, Durham, North Carolina, USA

² Duke Global Health Institute, Duke University, Durham, North Carolina, USA

³ Global Health Center, Duke Kunshan University, Kunshan, China

⁴ Department of Pathology, School of Medicine, Duke University, Durham, North Carolina, USA

⁵ Division of Pulmonary, Allergy, and Critical Care Medicine, Duke University Hospital, Durham, North Carolina, USA

⁶ Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

Keywords: adenovirus, epidemiology, lung transplant, outbreak

© The Author(s) 2018. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

***Corresponding Author:**

Gregory C. Gray, MD, MPH, FIDSA, Duke University, DUMC Box 102359, Durham, NC 27710,

Tel: 919-684-1032, gregory.gray@dm.duke.edu

***Alternate Corresponding Author:**

Sarah E. Philo, MS

Tel: 248-880-9843, philos357@gmail.com

Accepted Manuscript

Abstract

Here we summarize an April 2016, seven-patient cluster of human adenovirus (HAdV) infections in a cardiothoracic surgery intensive care unit. We show that the patients were infected with a single HAdV21b type. Rapid HAdV typing diagnostics and effective antiviral interventions are needed for immunocompromised patients suffering from HAdV infections.

Accepted Manuscript

Introduction

Human adenoviruses (HAdV) were first identified in 1953 and since then, eight species (A–G) have been identified comprising at least 68 unique types.^{1,2} The viruses have been shown to be associated with a variety of clinical manifestations including febrile respiratory illness, gastroenteritis, cystitis, and encephalitis.^{2,3} Because HAdV diseases are highly contagious and the virus can remain viable outside the host for several days, they have the potential to cause local outbreaks in both immune competent and immune deficient populations.³ Severe HAdV infections are prevalent in patients receiving stem cell or organ transplants as a result of their suppressed immune systems.^{3,4} In particular, lung transplantation patients are particularly susceptible to HAdV infections which can lead to severe pneumonia and mortality.⁴

When HAdV outbreaks occur in a clinical setting it is very important to investigate such that future outbreaks might be prevented. One seeks to understand if the outbreak is due to a single clone or multiple virus strains, and if the virus or viruses were initially acquired from the community or nosocomially. Among transplant or other immunosuppressed patients, one also seeks to learn if the index patient may have experienced viremia from his or her latent infection, or from transplanted tissue.⁵

In this report, we summarize our investigation regarding a seven-patient cluster of infections in a cardiothoracic intensive care unit at Duke University Hospital (a 950-bed tertiary care hospital in Durham, NC) during a 3-week period in April 2016.

Methods

A case of HAdV infection in this cluster was defined as a patient admitted to the cardiothoracic surgery intensive care unit whose specimens were positive for HAdV B/E species (eSensor® Respiratory Viral Panel, GenMark Diagnostics, Carlsbad, CA) over a 3-week time span in Spring of 2016. An epidemiologic investigation was conducted to identify potential common exposures among cases, including ward locations, procedures, medication, and staff. Bronchoscopes used for sample collection were identified and their cleaning and high-level disinfection procedures were assessed. Finally, staff were interviewed to identify concerns related to adherence of infection prevention policies and environmental cleaning.

Bronchoalveolar lavage (BAL) samples are collected from all patients post-transplant. BAL samples from each patient in the outbreak that had been preserved at -80°C, were de-identified, and sent to the Duke One Health Research Laboratory for additional molecular characterization. Viral DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA). Conventional PCR amplification was carried out on extracted DNA using primers that target hyper-variable hexon regions (nucleotides positions 18784–19161 compared to the complete reference genome KF577595), as previously described.⁶⁻⁸ If initial PCR amplification did not result in a relatively clear band of expected molecular weight, it was considered as unsuccessful. Six of the samples were unsuccessful for fiber, and four of the samples were unsuccessful for hexon. We then attempted to propagate an aliquot of original lavage samples in A549 cells (ATCC, Manassas, VA) for 3 to 7 days or until cytopathic effect (CPE) was detected. From the supernatant, extracted DNA was again examined by HAdV hexon gene PCR assays. PCR products were then purified and sequenced using Sanger sequencing (Eton Bioscience, Inc., Raleigh, NC). All viral sequences were edited and assembled using Geneious R9.0.3 (Biomatters, Ltd). A total of six new hexon partial genome sequences were generated

from this study and deposited in GenBank (accession numbers: MH371128-MH371133, Table 1) In addition, a next-generation sequencing (NGS) method was used to obtain the complete HAdV genome of the first index patient OHT-006 using Illumina MiSeq platform (Illumina, Inc., San Diego, CA) using a Kapa Hyper prep kit with a 300bp insert for library preparation. The quality of the NGS reads was then checked using FastQC, followed by trimming using Trimmomatic and assembled in Geneious. The novel HAdV21 genome was deposited in GenBank (accession number: MF502426).

Phylogenetic analyses were performed using hexon gene and full genome datasets. To reconstruct the hexon gene phylogeny, the final dataset consists of 176 hexon sequences, including global sequences (representing species B, D and E) downloaded from NCBI GenBank. Maximum likelihood phylogeny was reconstructed using RAxML v8.0.14 as implemented in Geneious, and branch support was assessed using 1,000 nonparametric bootstrap replicates. Additionally, two full genome datasets of global HAdV species B (147 sequences) and HAdV21 (32 sequences) were used to reconstruct maximum likelihood phylogenies, as described above.

Results

Seven patients met the HAdV infection case definition: six were recent lung transplant recipients and one was on extracorporeal membrane oxygenation (ECMO) support for acute respiratory distress syndrome (ARDS). Among the lung transplant patients, the median time from transplant to positive culture was 3 days (range 1 to 13 days). No specific medical personnel or bronchoscopes were implicated in the investigation. Six of the seven patients had relatively few signs or symptoms and fully recovered without treatment or sequelae. One patient

with symptoms was given brincidofovir therapy and also fully recovered. A number of mitigation strategies were implemented in the unit once the HAdV outbreak was identified. They included a mandatory mask policy for all staff and visitors to the unit, restriction of visitors in the unit, cohorting of infected patients, use of gowns and gloves for care of immunocompromised patients, and enhanced environmental cleaning. A single additional case (BAL specimen was not available for typing) occurred 10 days after implementation of the above measures, with no further cases occurring thereafter. The average length of stay for lung transplant patients in 2016 was 37.2 days, with length of stay during this HAdV outbreak ranging from 13 to 109 days (Table 1). The 30-day survival of each of the patients found to have HAdV infection was 100%.

Our hexon phylogeny (Supplemental Fig. 1) of HAdV revealed the cluster's novel viruses belonged to human HAdV species B and they were clearly well-nested within the strongly supported monophyletic B21 lineage (BS ML=99%). The hexon gene of the seven novel viruses shared a high level of genetic similarities (nucleotide sequence identities: 97.5–99.9%) with the global HAdV21a and HAdV21b strains that have been circulating during 1978–2016.⁹ In addition, the virus strains exhibited 99.2%–100% nucleotide similarities among the seven lung-transplant patients, suggesting the HAdV21 outbreak likely emerged from a single source, possibly from the first index case. Although we obtained only one complete genome from the index patient, our full genome HAdV21 phylogeny (Supplemental Fig. 2) revealed that this isolate was most closely related to HAdV21b strains (BS ML =100%) that are co-circulating to-date. The index virus also shared a high level of genetic similarities with other HAdV21b strains (nucleotide sequence identities: 99.91–99.99%).

Discussion

Among the HAdV strains that are associated with respiratory disease, HAdV21 infections are relatively uncommon but have sporadically caused outbreaks.^{1,3} In our 2004 to 2006 US national study of clinical HAdV infections from 22 laboratories, we found a relatively low prevalence of HAdV21 (2.1%) among 2189 typed HAdV specimens, an increased risk (odds ratio 7.6, 95% confidence interval 2.6-22.3) of severe disease compared to the more common HAdV3 infections, and an increasing trend of HAdV21 detections over time.¹⁰ In a 2015 report, Kajon et al. reviewed the molecular epidemiology of HAdV21 strains infecting military personnel from 1997 to 2011, also documenting recent increases in detection.⁹

The different genotypes of HAdV21 strains may also be changing in prevalence over time. Kajon et al. used restriction enzyme digests to study 173 HAdV21s detected among US military personnel.⁹ In this work, the authors identified recent clusters of what they called HAdV21a and HAdV21b strains which differ from the prototypic HAdV21 strain (first detected in Saudi Arabia in 1956) which is now designated HAdV21p. Kajon et al. identified eight HAdV21 variants and noted a switch in most prevalent strains among US military personnel from HAdV21a to HAdV21b in 2007. HAdV21a genotype viruses have been reported to be associated with neuropathic and cardiopathic disease in Malaysia (1997) and severe pneumonia and deaths in Germany (2005–2013).^{11,12}

To our knowledge this is the first HAdV21 outbreak associated with immunocompromised patients in a US cardiothoracic surgery intensive care unit. We detected HAdV21b infection in at least seven patients within a short period of time. We showed that the HAdV21 strains from the patients were closely related to recently circulating HAdV21b strains. Although HAdV21b was detected in these patients, they did not all present with serious clinical symptoms. Other research suggests that up to 78% of lung-transplant patients are asymptomatic at the time of viral detection, which explains the relatively few symptoms of disease in study patients.¹³

Fortunately, effective infection control interventions likely stopped the spread of HAdV21 in this outbreak. Were such measures not conducted, HAdV strains could have spread much more widely among this large hospital's immunocompromised. The outbreak underscores how rapid HAdV typing diagnostics and effective antiviral interventions are needed for immunocompromised patients suffering from HAdV infections.

Accepted Manuscript

Acknowledgements

This study was supported by Professor Gray's Duke University-sponsored startup funds. We thank the clinical nursing staff, and the laboratory staff who supported this investigation. There are no conflicts of interest present with the authors.

Accepted Manuscript

References

1. Gray GC, Erdman DD. Adenovirus vaccines. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, eds. *Vaccines, 7th ed.* Philadelphia: Elsevier, Inc.; 2017:121-133.
2. Murray PR, Rosenthal KS, Pfaller MA. Adenoviruses. *Medical Microbiology*. 7th Edition ed. Philadelphia, PA: Elsevier; 2013.
3. Lion T. Adenovirus Infections in Immunocompetent and Immunocompromised Patients. *Clinical Microbiology Reviews*. 2014;27(3):441-462.
4. Lynch JP, 3rd, Fishbein M, Echavarría M. Adenovirus. *Semin Respir Crit Care Med*. 2011;32(4):494-511.
5. Bridges CB, Lim W, Hu-Primmer J, et al. Risk of influenza A (H5N1) infection among poultry workers, Hong Kong, 1997-1998. *J Infect Dis*. 2002;185(8):1005-1010.
6. Lu X, Erdman DD. Molecular typing of human adenoviruses by PCR and sequencing of a partial region of the hexon gene. *Archives of virology*. 2006;151(8):1587-1602.
7. Xu W, McDonough MC, Erdman DD. Species-specific identification of human adenoviruses by a multiplex PCR assay. *Journal of clinical microbiology*. 2000;38(11):4114-4120.

8. McCarthy T, Lebeck MG, Capuano AW, Schnurr DP, Gray GC. Molecular typing of clinical adenovirus specimens by an algorithm which permits detection of adenovirus coinfections and intermediate adenovirus strains. *J Clin Virol.* 2009;46(1):80-84.
9. Kajon AE, Hang J, Hawksworth A, et al. Molecular Epidemiology of Adenovirus Type 21 Respiratory Strains Isolated From US Military Trainees (1996-2014). *J Infect Dis.* 2015;212(6):871-880.
10. Gray GC, McCarthy T, Lebeck MG, et al. Genotype prevalence and risk factors for severe clinical adenovirus infection, United States 2004-2006. *Clin Infect Dis.* 2007;45(9):1120-1131.
11. Ooi MH, Wong SC, Clear D, et al. Adenovirus type 21-associated acute flaccid paralysis during an outbreak of hand-foot-and-mouth disease in Sarawak, Malaysia. *Clin Infect Dis.* 2003;36(5):550-559.
12. Hage E, Huzly D, Ganzenmueller T, Beck R, Schulz TF, Heim A. A human adenovirus species B subtype 21a associated with severe pneumonia. *J Infect.* 2014;69(5):490-499.
13. Humar A, Doucette K, Kumar D, et al. Assessment of adenovirus infection in adult lung transplant recipients using molecular surveillance. *J Heart Lung Transplant.* 2006;25(12):1441-1446.

Table 1. Characteristics of seven HAdV-B-positive lung-transplant patients during an adenovirus outbreak at Duke University Hospital, USA in April 2016. Hexon and full genome sequence data indicated the cluster of cases was due to a single type of HAdV-21b (Also see supplemental Figures 1-3).

Patients' specimens	Day of outbreak adenovirus first detected	Length of hospital stay (days)	Hexon gene	
			GenBank accession number	Type
OHT-006*	0	109	MF502426	Ad21b
OHT-004	7	--	MH371131	Ad21b
OHT-005	13	72	MH371132	Ad21b
OHT-001	19	13	MH371128	--
OHT-002	19	16	MH371129	Ad21b
OHT-007	19	109	MH371133	Ad21b
OHT-003	20	--	MH371130	Ad21b

*Only the index case yielded a full genome sequence