Published in final edited form as:

J Clin Virol. 2017 July ; 92: 53–55. doi:10.1016/j.jcv.2017.05.010.

The Role of Next Generation Sequencing in Infection Prevention in Human Parainfluenza Virus 3 Infections in Immunocompromised Patients

Atul Kothari,

Division of Infectious Diseases, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Mary J Burgess,

Division of Infectious Diseases, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Juan Carlos Rico Crescencio,

Division of Infectious Diseases, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Joshua L Kennedy,

Division of Allergy and Immunology, Department of Medicine and Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Jesse L Denson,

Department of Pharmaceutical Sciences, University of New Mexico College of Pharmacy, Albuquerque, NM, USA

Kurt C Schwalm,

Clinical Translational Sciences Center, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Ashley N Stoner,

Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

John C Kincaid,

Arkansas Children's Hospital, Little Rock, AR, USA

Faith E Davies, and

Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Corresponding Author: Atul Kothari, MD, Division of Infectious Diseases, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA, akothari@uams.edu, Ph: 312 623 4430.

Authorship: All authors had access to the data and played a role in writing this manuscript.

GENBANK ACCESSION NUMBERS

All isolates were submitted to GenBank under accession numbers MF034888-MF034896

Conflict of Interest: All authors report no conflicts of interest relevant to this article

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Darrell L Dinwiddie

Clinical Translational Sciences Center, Department of Pediatrics, University of New Mexico Health Sciences Center, Albuquerque, NM, USA

Abstract

Background—Respiratory viral infections are a significant problem in patients with hematologic malignancies. We report a cluster of HPIV 3 infections in our myeloma patients, and describe the utility of next generation sequencing (NGS) to identify transmission linkages which can assist in infection prevention.

Objectives—To evaluate the utility of NGS to track respiratory viral infection outbreaks and delineate between community acquired and nosocomial infections in our cancer units.

Study Design—Retrospective chart review conducted at a single site. All patients diagnosed with multiple myeloma who developed symptoms suggestive of upper respiratory tract infection (URTI) or lower respiratory tract infection (LRTI) along with a respiratory viral panel (RVP) test positive for HPIV 3 between April 1, 2016, to June 30, 2016, were included. Sequencing was performed on the Illumina MiSeq™. To gain understanding regarding community strains of HPIV 3 during the same season, we also performed NGS on HPIV3 strains isolated from pediatric cases.

Results—We saw a cluster of 13 cases of HPIV3 infections in the myeloma unit. Using standard epidemiologic criteria, 3 cases were considered community acquired, 7 cases developed infection during treatment in the cancer infusion center, while an additional 3 developed infections during hospital stay. Seven patients required hospitalization for a median duration of 20 days. NGS enabled sensitive discrimination of the relatedness of the isolates obtained during the outbreak and provided evidence for source of transmission. Two hospital onset infections could be tracked to an index case; the genome sequences of HPIV 3 strains from these 3 patients only differed by a single nucleotide.

Conclusions—NGS offers a significantly higher discriminatory value as an epidemiologic tool, and can be used to gather real-time information and identification of transmission linkages to assist in infection prevention in immunocompromised patients.

Keywords

HPIV3; Next Generation Sequencing; Infection Control

Background

Respiratory viral infections (RVI) are a significant co-morbidity of hematologic malignancies and hematopoietic stem cell transplant (HSCT), affecting up to 30% of patients (1). Previous studies of patients in cancer units have shown outbreaks of respiratory syncytial virus (RSV) and human parainfluenza virus 3 (HPIV 3) (2–5). Describing the origins of infection using standard epidemiologic definitions of community versus nosocomial acquisition of infection is often challenging within this population, as many of these patients get a significant proportion of their care in outpatient infusion centers. However, being able to differentiate nosocomial infections from community acquired infections is crucial for the tracking hospital outbreaks and determination of the success of

infection control procedures. Our myeloma unit at the University of Arkansas for Medical Sciences (UAMS) conducts more than 400 autologous peripheral blood stem cell transplants (APBSCT) every year. We describe an outbreak of HPIV 3 infections in our myeloma unit in the summer of 2016, and describe the utility of next generation sequencing (NGS) in this setting.

Objective

To evaluate the utility of NGS to track RVI outbreaks and delineate between community acquired and nosocomial infections in our cancer units.

Study Design

This is a retrospective chart review conducted at a single site. This study was approved by the UAMS Institutional Review Board. All patients were diagnosed with multiple myeloma and had developed symptoms suggestive of upper respiratory tract infection (URTI) or lower respiratory tract infection (LRTI) along with a respiratory viral panel (RVP) test positive for HPIV 3 that was obtained for clinical reasons between April 1, 2016, to June 30, 2016, were included. Our molecular biology lab routinely uses a commercial multiplex respiratory virus PCR (Genbank Dx™) for standard clinical testing. Date of positive RVP was considered the date of diagnosis for the purposes of this study. Epidemiologic classification of cases as community onset, infusion center onset, and hospital onset infections were based on timing of the onset of symptoms and date of positive RVP for HPIV3 using a median incubation period of 3 days (6). For sequencing, RNA was isolated from all positive samples and analyzed for quality, and sequencing libraries were created. All samples were enriched using the UNM ResVir panel for 2×75bp sequencing on the Illumina MiSeq™. The Kraken metagenomic pipeline was used to identify viruses present. A custom CLC Genomics workflow was used to conduct sequencing alignment to viral references, generation of consensus genomes, variant detection, and phylogenetic analysis. In order to gain understanding regarding what strains of HPIV3 were circulating in the community during the same season, we also performed NGS on HPIV3 strains isolated from pediatric cases at Arkansas Children's Hospital.

Our infection control policy mandates institution of contact and droplet isolation for all patients with known RVIs admitted to hospital or in the outpatient cancer infusion center. All patients with multiple myeloma are admitted to a single unit in the hospital, and all hospital rooms are single patient rooms. In the outpatient infusion center, single patient rooms are used for patients with known RVIs. In order to further delineate the origins of infection and transmission chains of RVIs during viral outbreaks, we utilized NGS to obtain genome sequences of respiratory viruses.

Results

In the summer of 2016, we saw a cluster of 13 cases of HPIV3 infections in the myeloma unit at UAMS. Seven of the 13 patients were on intensive induction chemotherapy, 1 had undergone an autologous stem cell transplant and 5 were on maintenance chemotherapy.

Using standard epidemiologic case definitions, 3 cases were considered community acquired, while 10 cases appeared nosocomial (7 developed infection during treatments in the cancer infusion center and 3 were acquired after admission to the hospital). 46% of patients were febrile, 6 patients went on to develop lower respiratory tract infections. Antibiotics were prescribed in 92% of the patients. Seven patients required hospitalization; the median duration of hospitalization was 20 days with a standard deviation of 10.9 days. One patient received intravenous immunoglobulin secondary to severe respiratory symptoms. We evaluated the 13 clinical nasopharyngeal samples from these patients using a high throughput RNA enrichment method for viral genomes followed by NGS. This process confirmed HPIV3 infections in all 13 patients and generated high genome coverage (>97%) in 6 of the isolates.

Genome sequencing of these 6 strains enabled sensitive discrimination of the relatedness of the isolates obtained during the outbreak and provided evidence for source of transmission (Figure 1A, Table 1). Isolate UAMS 030, a community acquired infection, was closely related to a community acquired pediatric HPIV3 infection (ACRI 123). UAMS 034 & UAMS 038 developed infection in the cancer infusion center; but these were also closely related to two pediatric community strains (ACRI 108 & ACRI 106), suggesting acquisition in the community. Patient UAMS 047 (index case) developed infection in the community and was admitted for chemotherapy and treatment of pneumonia. He was placed on droplet and contact precautions on admission. UAMS 049 and 050 were also admitted for chemotherapy, but developed HPIV 3 infection during their hospital stay. Genome sequences of HPIV 3 from these 3 patients differed by only a single nucleotide and of note, patients UAMS 047 and UAMS 049 were in adjacent rooms (Figure 1B). Nursing and medical teams taking care of all 3 patients were similar.

Discussion

A recent large single center study had reported a reduction of RVIs in their HSCT unit from 10.3% to 4.4% by mandating universal surgical masks for all individuals in direct contact with HSCT patients in addition to standard infection control precautions (7). Of note, almost all the reduction in RVIs came from reduced incidence of HPIV3 infections with no significant reductions in the incidence of influenza A, B and respiratory syncytial virus (RSV) infections. In our study, NGS provided virologic evidence of transmission of HPIV 3 to 2 hospitalized patients from an index case who had developed infection in the community. As direct interaction between patients is limited in the hospital setting, we postulate that transmission in these 3 cases likely occurred via healthcare workers.

Our experience in patients with multiple myeloma undergoing autologous stem cell transplant or other forms of chemotherapy suggests improvement in infection control might decrease healthcare acquired cases of HPIV3. Currently, hospital infection control policies for respiratory viral pathogens include droplet and contact precautions. We believe that different respiratory viruses may have varying potential for nosocomial transmission. NGS combined with clinical epidemiologic data will provide a greater insight into the potential for different respiratory viral pathogens to spread within a nosocomial environment. We

have previously reported presence of multiple different strains of RSV during an outbreak using the same sequencing technology (8).

In our experience, and as recently reported, NGS offers a significantly higher discriminatory value as an epidemiologic tool, and can be used to gather real-time information and identification of transmission linkages to assist in infection prevention in these highly immunocompromised patients (9,10). A challenge going forward will be developing bioinformatics tools which will allow this sequencing information to be available to clinical providers in a rapid and actionable timeframe.

Acknowledgments

We thank Mona K Blacklaw, MT (ASCP), MB^{CM} Molecular Diagnostics Lab, UAMS and Frankie C Wolfe, RN, CIC Infection Control, UAMS for their assistance in the project.

Funding Source: Funding, including the efforts of Darrell L. Dinwiddie, was provided by HHS | NIH | National Center for Advancing Translational Sciences (NCATS) (UL1TR000041 and KL2TR000089). Dr. Kennedy has support from the NCATS (UL1TR000039, KL2TR000063), NIH National Institute of Allergy and Infectious Disease (K08AI121345), and the Arkansas Biosciences Institute.

References

1. Martino R, Porras RP, Rabella N, et al. Prospective study of the incidence, clinical features, and outcome of symptomatic upper and lower respiratory tract infections by respiratory viruses in adult recipients of hematopoietic stem cell transplants for hematologic malignancies. *Biol Blood Marrow Transplant.* 2005; 11:781–96. [PubMed: 16182179]
2. Ustun C, Slaby J, Shanley RM, et al. Human parainfluenza virus infection after hematopoietic stem cell transplantation: risk factors, management, mortality, and changes over time. *Biol Blood Marrow Transplant.* 2012; 18:1580–8. [PubMed: 22531491]
3. Nichols WG, Erdman DD, Han A, Zukerman C, Corey L, Boeckh M. Prolonged outbreak of human parainfluenza virus 3 infection in a stem cell transplant outpatient department: insights from molecular epidemiologic analysis. *Biol Blood Marrow Transplant.* 2004 Jan; 10(1):58–64. [PubMed: 14752780]
4. Jalal H, Bibby DF, Bennett J, Sampson RE, Brink NS, et al. Molecular Investigations of an Outbreak of Parainfluenza Virus Type 3 and Respiratory Syncytial Virus Infections in a Hematology Unit. *J Clin Microbiol.* Jun; 2007 45(6):1690–1696. [PubMed: 17392447]
5. Chu HY, Englund JA, Podczervinski S, et al. Nosocomial transmission of respiratory syncytial virus in an outpatient cancer center. *Biol Blood Marrow Transplant.* 2014; 20(6):844–51. [PubMed: 24607551]
6. Lessler J, Reich NG, Brookmeyer R, et al. Incubation periods of acute respiratory viral infections: a systematic review. *Lancet Infect Dis.* 2009;291–300. [PubMed: 19393959]
7. Sung AD, Sung JAM, Thomas S, Hyslop T, Gasparetto C, et al. Universal Mask Usage for Reduction of Respiratory Viral Infections After Stem Cell Transplant: A Prospective Trial. *Clin Infect Dis.* 2016; 63(8):999–1006. [PubMed: 27481873]
8. Kothari A, Kennedy JL, Hardin O, Burgess MJ, Rico Crescencio JC, et al. An Outbreak of Respiratory Syncytial Virus Infections in an Outpatient Cancer Unit: Clinical Characteristics and Molecular Investigations. *Open Forum Infect Dis.* 2016; (suppl 1)doi: 10.1093/ofid/ofw194.16
9. Greninger AL, Zerr DM, Qin X, Adler AL, Sampoleo R, et al. Rapid metagenomic next-generation sequencing during an investigation of hospital-acquired human parainfluenza virus 3 infections. *J Clin Microbiol.*
10. Houldcroft CJ, Beale MA, Breuer J. Clinical and biological insights from viral genome sequencing. *Nat Rev Microbiol.* 2017 Jan 16.doi: 10.1038/nrmicro.2016.182

Highlights

- Differentiating between community and nosocomial acquisition of infection is crucial
- Next generation sequencing can assist in tracking hospital outbreaks
- Parainfluenza 3 is an important cause of nosocomial outbreaks in cancer patients

Figure 1.

Tree indicating genome relatedness of the 6 HPIV3 isolates (>97% genome coverage) obtained by Jukes-Cantor neighbor joining with 1,000 bootstraps (A). Isolates obtained from adults are shown as circles and pediatric isolates are shown as diamonds. Community onset cases (blue), infusion center cases (yellow), and hospital onset cases (red). Timeline of the hospital transmitted cases (B).

Table 1

Characteristics of Adult HPIV3 infections

Isolate	Cohort	Infection Acquisition	Date of Symptom Onset/ Sample Collection	Date of Hospital Admission	URI/LRTI*
UAMS 030	Adult/Myeloma	Community	5/20/2016	N/A	URI
UAMS 034	Adult/Myeloma	Nosocomial (Cancer Infusion Center)	5/26/2016	N/A	URI
UAMS 038	Adult/Myeloma	Nosocomial (Cancer Infusion Center)	6/7/2016	N/A	URI
UAMS 047	Adult/Myeloma	Community	6/4/2016	6/4/2016	LRTI
UAMS 049	Adult/Myeloma	Nosocomial (Hospital Acquired)	6/21/2016	6/9/2016	LRTI
UAMS 050	Adult/Myeloma	Nosocomial (Hospital Acquired)	6/24/2016	6/8/2016	URI

* URI= Upper Respiratory Tract Infection, LRTI=Lower Respiratory Tract Infection