Journal of Clinical Virology 94 (2017) 37–41

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Limited diagnostic value of a multiplexed gastrointestinal pathogen panel for the detection of adenovirus infection in an oncology patient population

Tracy McMillen[a], Yeon Joo Lee[c], Mini Kamboj[b,c], N. Esther Babady[a,c,*]

[a] Clinical Microbiology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[b] Department of Laboratory Medicine and Infection Control Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States
[c] Infectious Disease Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States

ARTICLE INFO

Keywords:
Adenovirus
Genotypes
Gastroenteritis
Multiplexed molecular panel
Cancer patients

ABSTRACT

Background: Diagnosis of Adenovirus infections in transplant patients may be accomplished using either plasma or stool samples. IVD-cleared multiplexed gastrointestinal (GI) PCR panels offer an option for rapid testing of stool samples but most only target Adenovirus (HAdV) types F40/41.
Objectives: Given the potential significance of a positive adenovirus test in an immunocompromised patient, we sought to determine the frequency of type 40/41 in our patient population and the utility of a readily available multiplexed, FDA-cleared GI Panel for the detection of adenovirus infections.
Study design: A total of 215 specimens from immunocompromised patients mostly with hematologic malignancy or transplant recipients were evaluated including 107 plasma samples, 85 stool samples and 23 respiratory samples. Genotyping was performed successfully on 122 specimens.
Results: The most common type detected in all samples including stools was Adenovirus C/2. In a subset of patients with multiple specimen types tested, similar types were detected in all samples.
Conclusions: Although Adenovirus F40/41 is the most common enteric type, Adenovirus C/2 was the most common type identified in stools and subsequently plasma samples of our patient population. Implementation of assays that have wide reactivity for most adenovirus types is essential for optimal diagnostic yield.

1. Background

Human adenoviruses (HAdV) are double-stranded, non-enveloped DNA viruses that are responsible for a wide range of clinical syndromes including upper respiratory tract infection, pneumonia, nephritis, hemorrhagic cystitis, hepatitis and colitis. The severity of the infection caused by HAdV differs between patient populations; ranging from asymptomatic, self-limited illness to severe and life-threatening disseminated disease. Seven species (A–G) and 67 types of HAdV have been characterized based on serological techniques and/or sequencing methods [1].

Clinical disease syndromes associated with HAdV infections occur after primary infection or from reactivation of latent virus. HAdV establish long term latency in the lymphoid cells of various tissues and can reactivate under conditions of immunosuppression [1]. Further, predilection for latency in certain organ systems and resulting diseases is influenced by the infecting HAdV type. Among highly immunocompromised patients such as recipients of solid organ and hematopoietic stem cell transplants (HSCT), HAdV infection can have rapidly progressive and devastating consequences. In the era of effective antivirals and virus specific cytotoxic T cell therapy,diagnosis of HAdV gastroenteritis during periods of intense immunosuppression may also identify those at risk for invasive infections [2,3].

HAdV species F, type 40/41 (HAdV F40/41) have tropism for the gastrointestinal tract and are among the most common HAdV isolated from patients with community onset gastroenteritis. The GI tract (stools) is also the site adenoviruses are most frequently isolated from immunocompromised patients. Recently, several multiplex panels have become commercially available for detection of HAdV from GI and respiratory tracts [4]. While the respiratory panels are designed to detect a wide range of types, the GI panels only target HAdV F40/41. Given the potential significance of a positive HAdV test in immunocompromised patients, we sought to determine the utility of multiplexed GI panels by investigating the frequency of type 40/41 in stool samples of our patient population. We additionally characterized the diversity of types present in plasma and respiratory specimens collected during the same time period.

* Corresponding author.
E-mail address: babadyn@mskcc.org (N.E. Babady).

http://dx.doi.org/10.1016/j.jcv.2017.07.002
Received 11 November 2016; Received in revised form 30 June 2017; Accepted 3 July 2017

2. Objectives

The study evaluated the diagnostic utility of a multiplexed GI PCR panel in detecting HAdV from high risk patients with cancer.

3. Study design

3.1. Clinical specimens

This was a retrospective study conducted on specimens collected from patients at Memorial Sloan Kettering Center (MSKCC), a 470-bed tertiary cancer care center in New York City. Specimens tested included available left-over, respiratory (nasopharyngeal swabs and bronchial washings), plasma and stool samples. Plasma and stool samples included primarily positive specimens submitted for detection of HAdV by PCR between 2012 and 2016 in both pediatric and adult patients with cancer. Stool and respiratory samples were from patients with acute gastroenteritis or respiratory infections symptoms respectively while plasma samples were collected for diagnosis of disseminated diseases or monitoring of viral loads. Additional negative specimens were selected as part of the validation of the laboratory-developed assay used in the study. Repeat specimens from the same patient obtained on different days were included. Specimens were stored at −80 °C until testing.

3.2. Nucleic acids extraction

Extractions were performed on either the MagNA Pure Compact (Roche, Indianapolis, IN) or the EasyMag (bioMérieux, Durham, NC) depending on the number of samples to be tested. For plasma and respiratory samples, samples were briefly vortexed prior to extraction and 5 µL of MHV (Mouse Hepatitis Virus) DNA Processing Control (Luminex Corporation, Austin, TX) was added to 200 µL of each sample. Stool samples were processed by mixing 1000 µL of EasyMag Lysis Buffer reagent to Precellys® lysis beads (Caymen Chemical, Ann Arbor, MI) and approximately 100–150 mg of solid stool (or 100–150 µL of liquid stool). Samples were vortexed and incubated at room temperature for 13 min. Samples were then centrifuged at 13,200 rpm for 2 min. 200-µL of supernatant was used for the extraction along with the addition of 5 µL of the MHV processing control. Extraction was performed using the Total_NA_Plasma_100_400_V3 on the MagNA pure compact and the STOOL D 2.0.1 (Specific A 1.0.2) for stools and Generic 2.0.1 for all other sample types on the EasyMag.

3.3. Multiplex gastrointestinal pathogen panel (GPP)

The xTAG GPP assay (Luminex Corp., Austin, TX) was performed on stool samples following the manufacturers' instructions. The GPP assay targets Adenovirus F40/41.

3.4. Multiplex respiratory pathogen panel (RPP)

The BioFire RPP assay (bioMérieux, Durham, NC) was performed on respiratory samples following the manufacturers' instructions. The RPP assay target Adenovirus species A, B, C, D, E, and F.

3.5. Adenovirus real-time PCR

A laboratory-developed Adenovirus PCR using Luminex, MultiCode analyte-specific reagents (ASR) was performed on plasma and stool samples. Reactions were carried out in a final reaction volume of 25-µL including 20-µL mix that contained 1 unit of uracile-N-glycosylase, 1x ISOlution (Luminex), 1x Adenovirus primers pair (Luminex), 1x MHV Control primers (Luminex), 2.5 mM MgCl (Qiagen, Hilden, Germany), 1x Titanium Taq (Clontech, Mountain View, CA), PCR grade water (Roche, Basel, Switzerland) and 5 µL of extracted DNA. Real-time PCR was performed on the ABI 7500 (Applied Biosystems Foster City, CA) with the following amplification process of 1 cycle at 40 °C for 10 min, 1 cycle at 95 °C for 10 min, followed by 45 cycles at 95 °c for 5 s, 58 °C for 10 s, and 72 °C for 35 s. A melt curve stage was performed at 95 °C for 15 s, 60 °C for 1 min, 95 °C for 30 s and lastly 60 °C for 15 s.

Table 1
Distribution of specimens tested and genotyped for Adenovirus.

SPECIMEN TYPE	TOTAL NUMBER	POSITIVE SAMPLES	GENOTYPED SAMPLES	GENOTYPES RECOVERED (n)
Plasma	107	94	53	A/12 (1) A/31 (8) B/11 (1) B/55 (3) C/1 (1) C/1/2[a] (6) C/2 (18) C/5/6[a] (2) C/6 (2) D (1) D/64/19[a] (2) F/41 (8)
Stool	85	55	48	A/31 (5) B/3 (2) B/11 (2) C/1/2 (5) C/2 (12) C/5/6[a] (3) C/6 (3) D (2) D/17/49[a] (1) D/23 (1) D45 (1) D/64/69[a] (1) E/4 (2) F/41 (8)
Respiratory (NPS, BAL/BW)	23	23	21	A/31 (1) B/3 (2) B/35/11[a] (1) C/1 (2) C/2 (13) D/64/19[a] (1) E/4 (1)

Abbreviations: NPS, nasopharyngeal swab; BAL, bronchoalveolar lavage; BW, bronchial wash.
[a] Unable to distinguish between two serotypes.

3.6. Adenovirus sequencing

Genotyping of HAdV positive samples was performed as described by Moyo and colleagues using dual primers for sequencing [5]. The PCR reaction was performed in a final reaction volume of 15 µL that contained 3 µL master mix reagent (PerfeCTa® PreAmp 5X SuperMix, Quanta Biosciences), forward primer (10 µM); reverse primer (10 µM), PCR grade water (Roche, Basel, Switzerland) and 5 µL of extracted DNA. PCR products were purified using ExoSAP and sequencing was performed at the MSKCC Genomic Core facility as previously described [6]. HAdV genotypes were identified by comparing the nucleotide sequences to known HAdV sequences in the GenBank database using the NCBI Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A subset of samples was sequenced using a method described by Lu and Erdman [7], that targets a larger region of the hexon gene.

3.7. Statistical analysis

Statistical analysis was performed using the Fisher's exact test (GraphPad software, LA Jolla, CA).