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Diagnosis of Pediatric Acute Adenovirus Infections: Is a Positive PCR Sufficient?

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Abstract

Background—Human Adenovirus (HAdV), especially species C (HAdV-C), can be detected incidentally by PCR in nasopharyngeal (NP) samples, making it difficult to interpret clinical significance of a positive result. We classified patients into groups based on HAdV culture positivity from respiratory specimens, and the presence of an identified co-pathogen. We hypothesized that HAdV-C would be over-represented and viral burden would be lower in patients most likely to have incidental detection (i.e, with a negative viral culture and documented co-pathogen).

Methods—Immunocompetent children with HAdV+ NP specimens were classified into 4 Groups: Group I (HAdV culture (+) and no co-infection), Group II (culture (+) and co-infection), Group III (culture (−) and no co-infection), and Group IV (culture (−) and co-infection). Viral burden (Ct) and species were compared among Groups.

Results—Of 483 NP specimens, HAdV was isolated in culture in 252 (52%); co-infection was found in 265 (55%) patients. Group I (most consistent with acute disease) had significantly lower Cts (median 23.9 [IQR 22.2–28.1]) compared with Group IV (most consistent with incidental detection, median 37.3 [IQR 35.3–38.9], p <0.0001). HAdV-C accounted for 41% samples of Group I and 83% of Group IV (p <0.0001). We identified a subset of 22 patients with bacterial or fungal co-pathogens, 18 of whom had no growth on viral culture (Group IV) with a median Ct of 37.4 (IQR 33.9–39.2).

Conclusions—Species identification and viral burden may assist in interpretation of a positive HAdV result. Low viral burden with HAdV-C may be consistent with incidental detection.

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Keywords

Adenovirus; PCR; viral burden; Ct; adenovirus species; pediatrics; viral co-infection; bacterial co-infection

Introduction

Human adenoviruses (HAdV) account for 7–8% of viral respiratory illnesses in children less than 5 years[1–3]. HAdV infections can cause prolonged fever with elevated inflammatory markers[4] and may mimic other illnesses that require specific treatment, such as bacterial infections or Kawasaki disease (KD)[3–6]. Over 60 HAdV types have been defined based on genomic sequences and are classified into 7 species (A–G). HAdV-C is known for its ability to establish persistence in lymphoid organs such as tonsils and adenoids[7–9].

Polymerase chain reaction (PCR) has become the standard diagnostic method for HAdV detection. It provides timely results with superior sensitivity to conventional methods. However, several aspects of molecular detection of HAdV make interpretation of a positive result from a nasopharyngeal (NP) specimen challenging. First, HAdV can be detected by PCR in the NP of up to 11% of healthy, asymptomatic children[10–13], compared with the lower detection rate (0.6%) by culture[3]. Second, prolonged intermittent PCR detection of the same HAdV strain from NP secretions after primary infection has been described[14]. Third, a clinically relevant PCR for positivity has yet to be established[15]. Misattribution of the etiology of disease based on molecular detection of HAdV in respiratory specimens[5, 12, 16] may have clinical consequences (e.g., coronary aneurysm because of delayed treatment for KD; or lack of treatment for serious bacterial infections in febrile infants). Accurate diagnosis of acute HAdV-associated disease is also crucial for evaluation of new antiviral treatments.

Because there is no practical gold standard for assessing whether the detection of HAdV in respiratory specimens using PCR is *causal or incidental*, we classified patients into 4 groups to assess for likelihood of HAdV causality of disease based on: 1) the ability to recover *infectious* HAdV in culture from their respiratory specimens, and 2) the presence of a simultaneously identified co-pathogen (viral, bacterial, or fungal) which may explain their clinical symptoms alternatively. Patients with sole HAdV detection and positive HAdV culture were considered most likely to have acute HAdV disease, while those with other co-pathogens and negative HAdV culture were considered least likely to have acute HAdV disease. We hypothesized that in cases most consistent with incidental detection, specimens would have a lower viral burden, and a higher percentage of HAdV-C.

Methods

Study population: symptomatic patients

Patients <21 years with a HAdV PCR+ respiratory specimen from 8/2011–12/2012 were identified from laboratory records. Patients were tested using the standard respiratory viral PCR panel (RVP) per clinician request at urgent care centers/emergency department or inpatient units. Subjects with underlying medical conditions were included, but those with

immunodeficiency or receiving immunosuppressive medications were excluded. Only the first HAdV+ respiratory specimen from an individual patient was included. The study was approved by the Nationwide Children's Hospital (NCH) Institutional Review Board.

Study population: asymptomatic, healthy children

Forty-eight NP swabs specimens from healthy children who were asymptomatic at least 2 weeks prior to enrollment were prospectively collected and tested for the standard RVP panel with IRB approval from (collected from 8/2010 to 1/2012) for respiratory viral testing (see below).

Clinical Laboratory PCR Testing

The RVP consists of separate laboratory-developed tests for detection of HAdV, human metapneumovirus (hMPV), parainfluenza viruses 1, 2 and 3 (PIV) and human rhinovirus/enterovirus (HRV/EV) using a real-time PCR format based on previous studies with slight modifications[17, 18, 19]. The Hologic ProFlu+ assay identified influenza A/B and respiratory syncytial virus (RSV). Total nucleic acid was extracted from 200 μL of sample using the NucliSENS EasyMag (bioMerieux, Burlington, NC), and eluted into 110 μL for analysis as above. Remnant specimens were stored at −80°C for further testing. The semi-quantitative HAdV real-time PCR was based the protocol developed by Heim et al.[5, 20] targeting a conserved region of the HAdV hexon gene and designed to detect all serotypes of HAdV. Samples were considered HAdV positive if threshold cycles (Ct, inversely correlated with viral burden) were ≤40.

HAdV Culture Isolation

At Lovelace Respiratory Research Institute (LRRI), clinical specimens were inoculated onto A549 cell monolayers for HAdV isolation[21–23]. Samples had undergone only one freeze-thaw cycle before inoculation to cell cultures.

HAdV Species Identification

For all specimens, direct molecular species identification (DMS) from the original respiratory specimen was performed at NCH (Fig.1B). Primer/probe sets that detect HAdV-A, C and D were described previously with modification[24]; primer/probe sets that detect HAdV-B, E and F were designed on the basis of the available HAdV DNA sequence information (NCBI database) (Supplemental Table 1). In addition, if HAdV was isolated in culture, species (and type) identification was performed at LRRI by restriction enzyme analysis of viral genomic DNA and by amplification and sequencing of hexon and fiber genes as previously described (Fig. 1B)[5, 21–23].

Housekeeping gene

In order to evaluate specimen quality, we examined the cellularity of the original respiratory samples by determining the relative level of a two-copy human house-keeping gene, Zink finger 80 (*ZnF*80, 3q13.31) in each sample[25].

Clinical phenotypes

Patients were classified using chart review based on their primary clinical syndrome at the time of testing for HAdV into clinical phenotypes:

1. **Fever alone** with no other specific signs or symptoms.
2. **Upper respiratory tract illness (URTI)** was defined as upper respiratory symptoms such as cough, coryza, conjunctivitis, pharyngitis with or without exudate, and/or pharyngoconjunctival fever.
3. **Lower respiratory tract illness (LRTI)** was defined as acute respiratory illness with symptoms or signs of lower airway involvement (e.g., dyspnea or tachypnea, hypoxia, and/or abnormal lung examination).
4. **Gastrointestinal illness** was defined as gastroenteritis (only if diarrhea was a predominant complaint) or radiologically proven intussusception.
5. **Sepsis-like syndrome** was defined as hemodynamic instability with greater than 1 organ dysfunction.
6. **Unclassified disease** was noted for patients with insufficient documentation to classify illness phenotype.

Adenovirus Group definitions

Patients were classified into 4 Groups based on the ability to recover HAdV in culture and co-infection status. Viral co-infection was defined as detection of a respiratory virus using standard RVP which was performed in all specimens. Bacterial co-infection was defined as bloodstream infection with a pathogenic organism, culture proven skin/soft tissue infection, urinary tract infection as defined by American Academy of Pediatrics[26], isolation or PCR detection of a pathogen in pleural fluid, or PCR NP detection of respiratory pathogens (*Mycoplasma pneumoniae* and *Bordetella pertussis*).

All patients had at least one symptom clinically compatible with HAdV-associated disease.

1. **Group I**: Patients with a PCR+ respiratory specimen, HAdV culture (+) and no identified co-pathogen.
2. **Group II**: Patients with a PCR+ respiratory specimen, HAdV culture (+) and a simultaneously identified co-pathogen (viral, bacterial, or fungal as previously described) which might also explain the clinical symptoms.
3. **Group III:** Patients with a PCR+ respiratory specimen, HAdV culture (−) and no identified co-pathogen.
4. **Group IV:** Patients with a PCR+ respiratory specimen, HAdV culture (−) and a co-pathogen which could account for the observed clinical symptoms alternatively.

For the purposes of this study, Group I was considered to be most consistent with acute HAdV disease and Group IV was considered to be most consistent with incidental detection.

Statistical analysis

Mann-Whitney or Kruskal-Wallis tests were used for comparisons between two or several groups as appropriate, and Chi square test was used for proportions. A two-tailed p value <0.05 was considered significant. All analysis, including receiver operating characteristic (ROC) analysis were performed using GraphPad Prism, (San Diego, CA).

Results

Adenovirus Case Classifications and Clinical Characteristics

A total of 7083 specimens (99% NP swabs, and 1% from eye, throat swabs or pooled specimens) were tested by RVP. HAdV was detected in 483 unique patients who were classified into 4 Groups according to our case definition (Fig.1A). The majority of patients (416, 86%) were < 5 years of age. Clinical characteristics of each Group are summarized in Table 1.

Adenovirus Cases and Ct values

Ct values vs. viral isolation rates—The relationship between HAdV Cts and culture isolation rates are shown in Fig. 2. HAdV Cts of culture (+) Groups (Groups I and II; median Ct 25.8 [IQR 22.5–29.9]) were significantly lower than those from culture (−) Groups (Groups III and IV; median 37.4 [IQR 35.2–38.8], p<.0001). Additionally, we compared clinical and virologic characteristics of the patients whose respiratory specimens were culture (+) and had low viral burden (HAdV Cts >30, n=62) from those with culture (−) and high viral burden (HAdV Cts ≤30, n=7). There was no difference in age, days of fever, clinical phenotype, Ct of housekeeping gene, co-infection status or predominance of one HAdV species between these two subpopulations.

Ct values in sole HAdV detection vs. Co-infection—HAdV Ct values for each Group according to case definitions are shown in Table 1. Cts in sole HAdV detection Groups (Groups I and III; median Ct 27.2 [IQR 23–34.6]) were significantly lower than those in co-infection Groups (Groups II and IV; median Ct 35.2 [IQR 30–38.1], p<.0001). Median Ct values for HAdV-C and non C species in each group are shown in Figure 3A and 3B. Because of the known high detection rate of HRV/EV among asymptomatic children, we examined the median HAdV Cts in the co-infection Groups (II and IV) among those in which the only co-infection was HRV/EV vs. other viral co-infections. There was no significant difference in median HAdV Cts among specimens with HRV/EV co-infection vs. co-infection with other respiratory viruses in Group II (median HAdV Ct 26.9 vs. 29.2, p=0.55). Similarly, there was no significant difference in median HAdV Cts among Group IV with co-infection with HRV/EV only vs. co-infection with other respiratory viruses (median HAdV Ct 37.2 vs 37.2, p>0.99).

HAdV Species Identification

Culture Based Species Identification (CBS)—HAdV-C and -B were identified in the majority (243/252, 96%) of CBS samples. In Group I, HAdV-B was predominant (53%), while HAdV-C was predominant (83%) in Group II (Table 1).

Direct Molecular Species Identification (DMS)—For specimens which were culture-negative and for which the only available species identification was via DMS (n=191), HAdV-C was predominant (n=159, 83%) followed by HAdV-F (n=23, 12%). Two or more HAdV species were identified in 6 culture negative specimens (Table 1).

Correlation between CBS and DMS—There was 100% concordance between results obtained by CBS and DMS for species identification. The DMS identified additional HAdV species in 8 culture positive specimens (Table. 1).

Human-House Keeping Gene

Median Ct values for the housekeeping gene for each Group were within 1 Ct difference of one another: Group I, 25.2 (IQR 24.2–26.4); Group II, 24.6 (IQR 23.6–26.1); Group III, 24.7 (IQR 23.9–25.6); Group IV, 25.1 (IQR 24.3–26.4).

Adenovirus detection In Asymptomatic, Healthy Children

Two (4%) out of 48 NP specimens from asymptomatic healthy children (median age 119 months, IQR 45.5–157.2 months) were positive for HAdV. One specimen was identified as HAdV-C with a Ct of 33.1 and the other was non-typeable with a Ct of 38.8. Neither subject had any co-detection of other respiratory virus.

Characteristics of Unique Patient Populations

Patients with identified viral co-infection—In the co-infection Groups (Groups II and IV, n=265), there were 239 specimens in which HAdV was co-detected with other respiratory viruses. The most frequent co-detections were HRV/EV (43%), RSV (42%), PIV (9%), hMPV (5%), and Flu A/B (3%).

Patients with identified bacterial and/or fungal co-pathogens—Twenty-two (8%) patients had concomitant bacterial and/or fungal infections identified at the time of HAdV detection (Table 2). Of 22 patients, 18 patients (81%; cases 1–18) were classified as Group IV with a median Ct of 37.3 (IQR 34–39). There were 4 patients with relatively lower Ct (median Ct 27.9) classified as Group II (cases 19–22). Of 22 patients, 10 patients had bacteremia/fungemia or urinary tract infection; all had high Cts ≥35 and HAdV-C was identified in all specimens when DMS was successful.

Infants younger than 60 days—A total of 9 patients were under 60 days at the time of HAdV detection with a median age of 37.4 days (IQR 25.1–43.9 days); 3 were late preterm infants (all gestational age of 36 weeks) and all infants were admitted secondary to respiratory symptoms (5 URTIs and 4 LRTIs). Only one patient (45 days old), who had HAdV-C (Ct 28.7) with HRV/EV and RSV co-infection, required ICU care with mechanical ventilator for 6 days due to LRTI. Sepsis-like syndrome was not observed in this small cohort. Two patients were classified to Group II (2 C), 3 to Group III (1 C, 1 F, 1 nontypeable), 4 to Group IV (3 F, 1 nontypeable) and overall median HAdV Ct was 38.1 (IQR 34.9–39.1).

Nasopharyngeal Ct values and ROC curve for growth in culture—To assess the relationship between semi-quantitative Ct values and the ability of specimens to grow in viral culture, an ROC curve was performed. Area under the ROC curve (AUC) for all Groups was 0.9549 (95% confidential interval (CI); 0.9362–0.9736, p <0.0001). When using only values from Groups I and IV, the AUC was 0.9827 (95% CI; 0.9707–0.9947, p <0.0001, Supplemental Figure 1A). We then performed a second ROC curve for the two most common species identified-HAdV-B and C. The AUC for species B was 0.9553 (95% CI; 0.9327–0.9778, p <0.0001) and HAdV-C was 0.9546 (95% CI; 0.9078–1.000, p <0.0001). ROC by subtype is shown in Supplemental Figure 1B.

Discussion

It is difficult to clearly distinguish incidental HAdV detection from acute HAdV associated disease in the absence of a longitudinal study. However, we were able to retrospectively classify patients based on virologic characteristics into categories that may shed light on the likelihood of HAdV attributable causality of disease. Since there is no established gold standard to diagnose acute HAdV disease, we utilized recovery of HAdV in culture as a marker of active disease because there is a much lower rate of asymptomatic detection in children using culture (0.6%)[3] versus PCR (3–11%)[10–12]. Thus, culture provides a higher positive predictive value for acute disease than PCR detection alone. We first identified Group I (most consistent with acute disease) and Group IV (most consistent with incidental detection) as the most straightforward categories. Group I patients most likely had acute HAdV disease as they had compatible illnesses and HAdV identified using two different methods (PCR and viral culture). Group IV patients had an alternate explanation for their symptoms (viral or bacterial/fungal co-pathogen) and their specimens failed to yield an HAdV isolate in culture, making it more likely that the detection of HAdV represented an incidental detection rather than the primary cause of acute symptoms, especially when a high Ct of HAdV-C was identified. Two virologic criteria, when used together, were helpful to discriminate these two Groups. First, Cts were significantly lower in Group I than in Group IV (median Cts: 23.9 vs. 37.3, p<.0001). Second, HAdV-C was overrepresented in Group IV (83%) compared to Group I (41%, p<.0001).

HAdV-C is indeed a common cause of primary HAdV disease in young children[3, 4, 27] but it is also known to be the most common HAdV species detected incidentally in pediatric tonsils and adenoids[7, 8, 28]. Specifically for HAdV-C, the semi-quantitative viral burden (Ct value) was helpful in distinguishing Group I from Group IV, as HAdV Cts were lower in Group I vs. Group IV (median Cts: 24.0 vs. 36.6, p<.0001). We also found differences in the positive predictive value of a given Ct for HAdV-B and C (the two most common HAdV species identified, Supplemental Figure 1B) for culture positivity, indicating that species and viral burden should be interpreted together; relatively higher Ct values still may be relevant for HAdV-B, but may not be as relevant for HAdV-C.

Although a similar significant difference in Cts was found between Groups II and III (median Ct 27.8 in Group II vs. 37.6 in Group III), the role of HAdV-C as the cause of disease was less clear in these two Groups. HAdV-C was also more predominant than HAdV-B in Group II (83%) with low Cts (median Ct: 28). Children in Group II were

relatively young (median 11.1 months). We believe that HAdV-C was more common in this group because it causes acute primary disease at a younger age than HAdV-B[4, 27] and co-infection with other respiratory viruses is more common in younger children[29, 30], indicating that true acute co-infection of HAdV-C with HRV/EV or RSV is clearly possible. Group III remains the most challenging group to interpret, as there are several possibilities: 1) a recent HAdV infection with low viral burden in the respiratory tract, 2) specimen obtained during incubation period, 3) low-level persistence during another illness, or 4) an antecedent HAdV illness followed by a presumed bacterial illness such as acute otitis media or pneumonia, as previously described[31–33].

One of the most concerning situations would be attribution of disease etiology to HAdV when there is a serious bacterial infection, so we examined this cohort specifically. The majority of these patients (81%) were classified as Group IV (most consistent with incidental detection). We found that HAdV semi-quantitative viral burden and species identification were especially helpful in the subgroup of patients with bloodstream or urinary tract infection (n=10, Table. 2). All of these patients' specimens had Cts ≥35 and all typeable samples were identified as HAdV-C by DMS. We also noted 9 patients who had fever alone as their clinical phenotype, and the majority had HAdV-C or F with high Cts (cases 1–9, Table 2). Although the clinical significance of NP detection of HAdV-F (types 40/41) is still unclear[34,35], it has been detected incidentally from pediatric respiratory samples[28]. Others have reported that HAdV can be associated with fever without localizing symptoms[12], however our data suggest that attributing febrile illness to HAdV alone, specifically when HAdV-C or F is detected in the NP with low viral burden, requires a cautious interpretation.

We identified 9 infants < 60 days with HAdV infection. HAdV has been detected in amniotic fluid or placental tissue, thus the source of infection may have been through vertical or horizontal transmission after birth[36, 37]. Although this particular population is at high risk for severe or disseminated HAdV disease[38], the majority of young infants (89%) did not demonstrate serious illness related to HAdV infection. The difference in this cohort vs. prior cohorts may be the method of identification of HAdV (highly sensitive PCR vs. viral culture/direct fluorescence antibody testing), the fact that the children were all term infants, or that the median age at the time of identification of HAdV infection was older than in prior cohorts. We also noted an over-representation of infections by HAdV-F (4 of 9 specimens, 44%) in young infants <60 days whose clinical phenotype was respiratory tract illness. Because HAdV-F is most frequently associated with gastro-intestinal illness, the clinical significance of detection of HAdV-F in infants with respiratory illness is unclear.

Our study has limitations. A negative HAdV culture does not necessarily exclude acute disease attributable to HAdV infection as it may be impacted by duration of illness at the time of sample collection. The retrospective clinical data collection did not allow for consistent documentation of exact duration of all symptoms, so we used timing of fever before HAdV testing to estimate the duration of illness. This study did not include longitudinal samples, so we could not evaluate the dynamics of HAdV detection during acute illness. Viral cultures were performed on stored frozen specimens and this could have impacted the preservation of virus infectivity, however we did note consistent cellularity of specimens using a human housekeeping gene, diminishing the likelihood that specimen

quality affected results. The A549 cell line used for viral isolation does not easily support growth of HAdV-F (types 40 and 41)[39], likely impacting results. However HAdV-F accounted for only 5% of the detected HAdV in the cohort. The specific Ct values calculated with our laboratory assay are not directly equivalent to those obtained with other PCR assays, but they are useful for improving the clinical interpretation of PCR-based HAdV testing.

The present data indicate that when PCR testing is used for diagnosis of HAdV infection and disease, there is a need for additional assays that provide both viral load quantitation and species identification to aid the interpretation of positive results. There are FDA-approved singleplex and multiplex PCR assays[40–44] for diagnosis of respiratory viruses, but no FDA-approved test provides comprehensive HAdV species identification and viral load.

In conclusion, detection of HAdV in pediatric NP samples by PCR-based methods does not necessarily establish causality of disease. Although detection of HAdV-C is associated with acute disease, if it is detected with a low viral burden (high Ct) clinicians should consider the possibility of incidental detection. Both HAdV typing and viral load quantitation may be useful tools to assess the clinical significance of HAdV detection and should be considered as new assays are developed to improve the diagnosis of HAdV infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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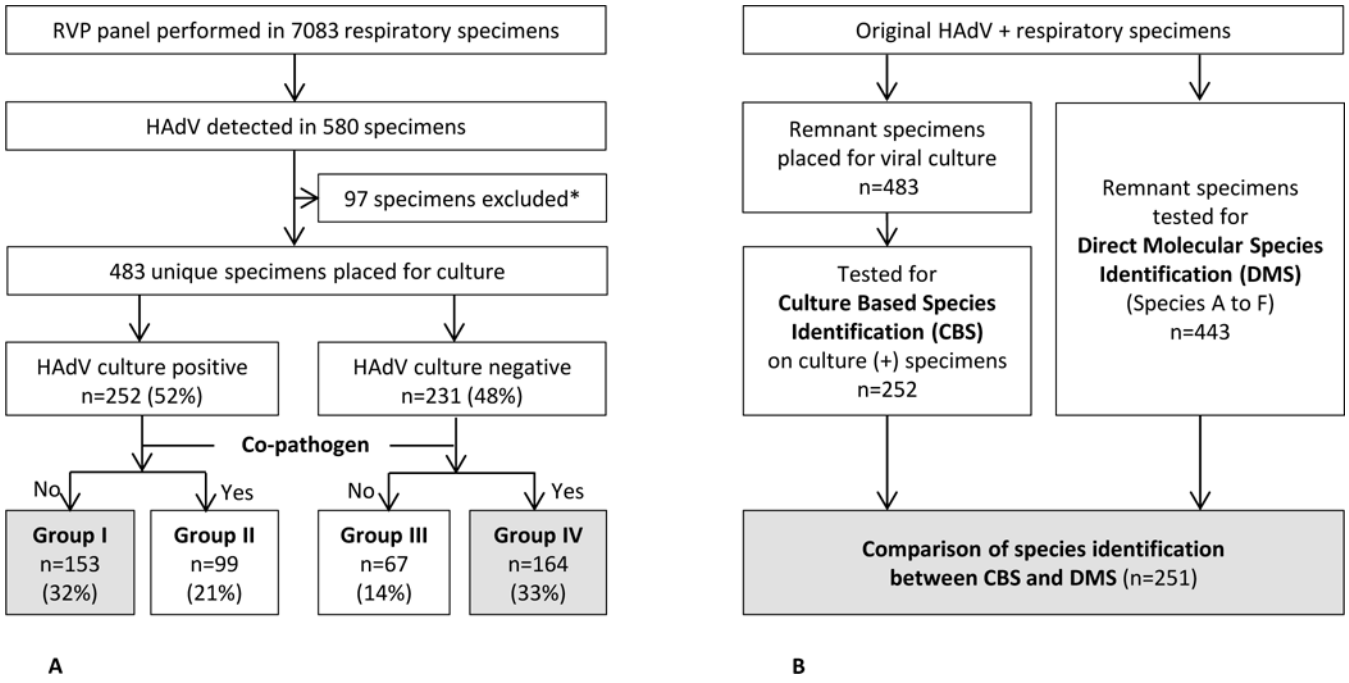

Figure 1.

A. Schematic Flowchart of Patient Enrollment and Case Classification

Group I was considered most consistent with acute HAdV associated infection and Group IV was considered most consistent with incidental detection of HAdV.

*32 patients had ≥ 2 episodes of HAdV detection during study period and 9 patients were immunocompromised hosts.

B. Schematic Flowchart of Testing for Species Identification

Abbreviations: RVP, respiratory virus PCR; HAdV, Human Adenovirus; CBS, Culture Based Species Identification; DMS, Direct Molecular Species Identification

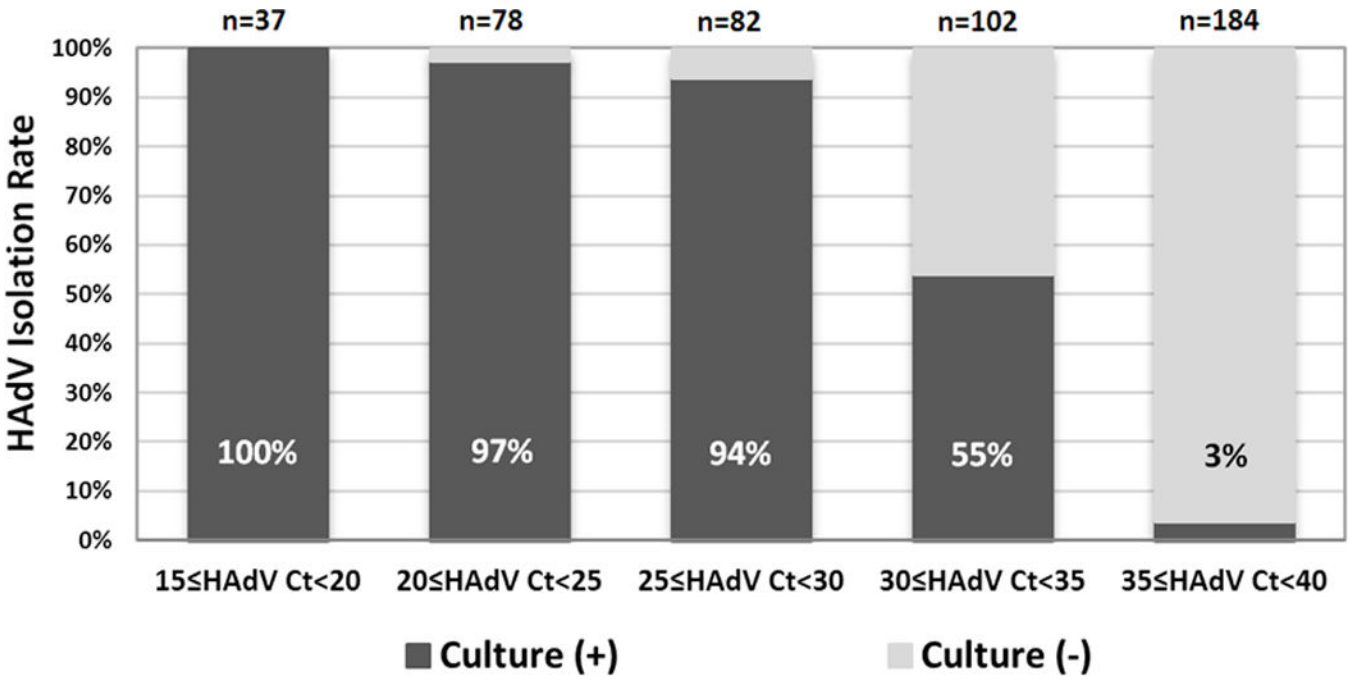

Fig 2. HAdV Ct values versus Isolation Rates

A total of 245 (97%) of specimens that were culture positive had a corresponding NP Ct of <35 ($p<0.0001$).

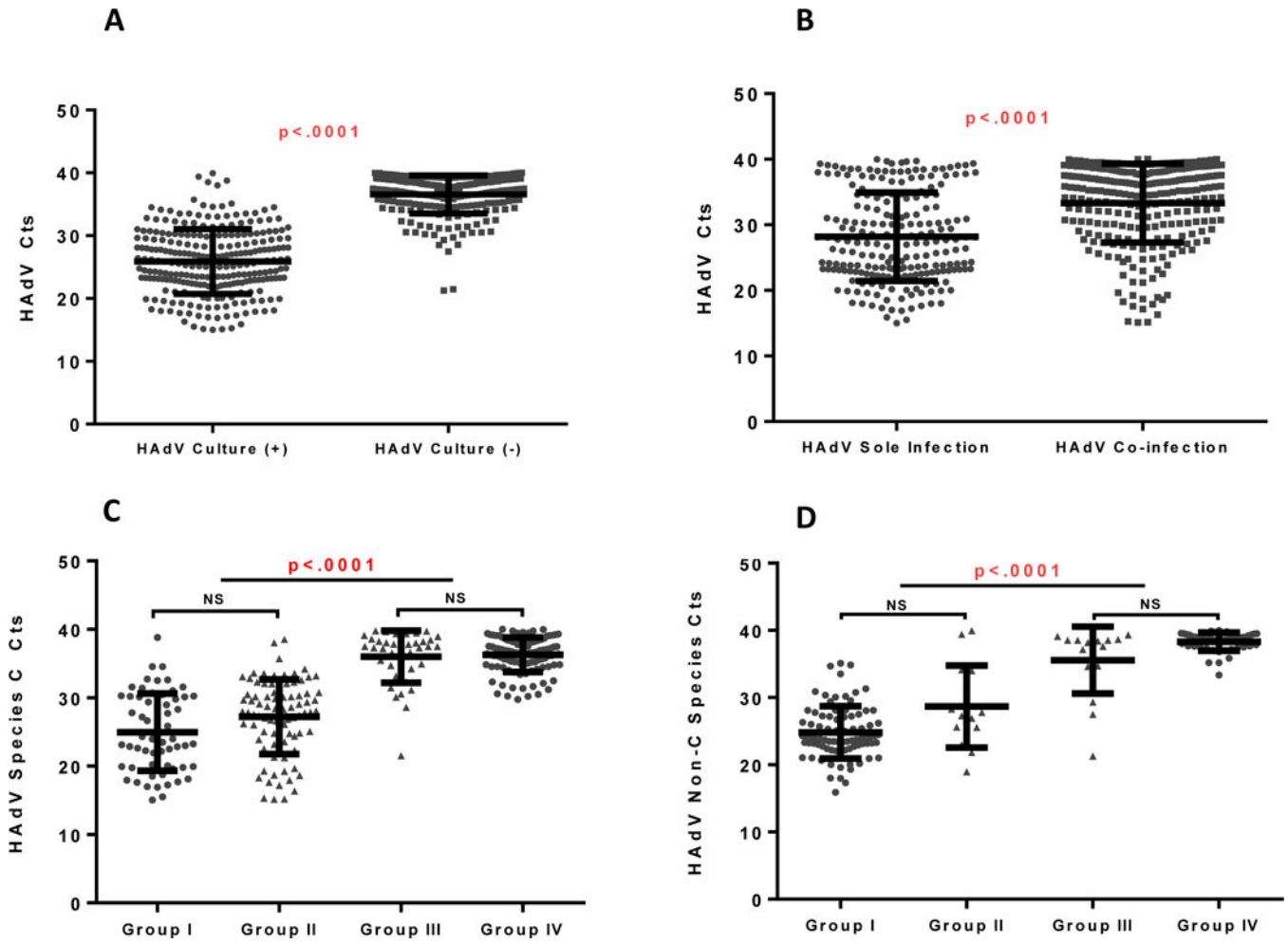

Figure 3.

A. Ct values of HAdV culture (+) group vs HAdV culture (−) group

Median Cts (IQR): HAdV culture (+) group 25.8 (22.5–29.9), HAdV culture (−) group 37.4 (35.2–38.8)

B. Ct values of HAdV sole infection group vs HAdV co-infection group

Median Cts (IQR): HAdV sole infection group 27.2 (23–34.6), HAdV co-infection group 35.2 (30–38.1)

C. Ct values of HAdV-C In Each Group

Median Cts (IQR) in each group: Group I 24.0 (19.9–30.1), Group II 28.0 (24.4–30.7), Group III 37.5 (34.2–38.5) Group IV, 36.6 (34.9–38.2)

D. Ct values of non HAdV-C Species By Group

Median Cts (IQR) in each group: Group I 23.8 (22.8–27.2), Group II 26.9 (25.2–33.9), Group III 38.2 (34.12–38.6), Group IV 38.1 (37.6–39.3)

Table 1

Characteristics of Adenovirus (HAdV) Patients by Group Classification

Case Classification	Total N=483	Group I Δ n=153 (32%)	Group II n=99 (21%)	Group III n=67 (14%)	Group IV Δ n=164 (33%)	*P**	*P* Δ
Clinical Characteristics							
Age (month, median) (IQR)	18.4 (8.4–38)	29.8 (11.3–57.6)	11.1 (5.9–20.3)	19.5 (8.4–36.7)	16.7 (8.3–31.4)	<.0001	<.0001
Male : Female ratio	1.3:1	1.1:1	1.7:1	1.8:1	1.2:1		
Underlying condition (%)	185 (38)	42 (27)	35 (36)	32 (48)	76 (46)	.002	.0008
Patient location							
Outpatient (%)	153 (32)	69 (45))	32 (32)	23 (34)	29 (18))	<.0001	<.0001
ICU (%)	91 (19)	8 (5	23 (23)	12 (18)	48 (29	<.0001	<.0001
Length of stay (median days)	2	2.2	2.9	2.5	2.5	NS	NS
Patients with fever (%)	335 (69)	137 (90)	65 (65)	44 (66)	89 (54)	<.0001	<.0001
Duration of fever (median days prior to HAdV testing)	2	3	2	1.5	2	.003	.013
Clinical Phenotype (%)							
URTI	223 (46)	106 (69)	39 (39)	36 (54)	42 (26)		
LRTI	204 (42)	24 (16)	53 (54)	23 (35)	104 (63)	<.0001	<.0001
Fever alone	27 (6)	10 (7)	1 (1)	3 (4)	13 (8)		
Others[a]	29 (6)	13 (8)	6 (6)	5 (7)	5 (3)		
Co-pathogen (%)	265 (55)						
Virus only	243 (92)	NA	95 (96)	NA	146 (89)		
Bacteria and/or fungus	22 (8)	NA	4 (4)	NA	18 (11)		
Virologic Characteristics							
HAdV Cts, median (IQR)	32.1 (25.1–37.4)	23.9 (22.2–28.1)	27.8 (24.7–31.7)	37.6 (34.5–38.5)	37.3 (35.3–38.9)	<.0001	<.0001
HAdV species (%)							
C	307 (67)	65 (41)	83 (83)	46 (82)	113 (83)		
B	103 (21)	81 (53)	14 (14)	3 (5)	5 (4)		
F	26 (5)	3 (2)	0 (0)	4 (7)	19 (14)	<.0001	<.0001
Others (A, E or D)	21 (4)	10 (7)	3 (3)	3 (5)	5 (3)		

Case Classification	Total N=483	Group I $^{\Delta}$ n=153 (32%)	Group II n=99 (21%)	Group III n=67 (14%)	Group IV $^{\Delta}$ n=164 (33%)	*P* *	*P* $^{\Delta}$
2 species	14 (3)	6[b] (4)	2[c] (2)	0 (0)	6[d] (4)		
NA[e]	40	0	1	11	28		

Abbreviation: HAdV, Human Adenovirus; URTI, upper respiratory tract illness; LRTI, lower respiratory tract illness; Ct, cycle threshold; IQR, interquartile range; NA, not applicable

[a] 12 with GI illness (9; gastroenteritis, 3; intussusception), 2 with sepsis like syndrome, 15 unclassified

[b] 3 C+F, 2 B+C, 1 E+C,

[c] 2 C+A,

[d] 1 B+D, 2 C+F, 1 B+C, 2 B+C

[e] Specimens could not be identified by DMS or specimen could not be found for DMS (n=1).

[*] Kruskal-Wallis Test or Chi-square test for all Groups

[Δ] Mann-Whitney Test, Chi-square test or Fisher's exact test for Groups I and IV

Table 2

Adenovirus (HAdV) Cases with Identified Bacterial and/or Fungal Co-pathogens

	Clinical Characteristics				Viral Characteristics		Copathogen	
	Age(month)/Sex	**Underlying Conditions**	**Clinical Phenotype**	**Case Classification**	**HAdV Cts**	**HAdV Species**	**Bacterial/Fungal organism**	**Source**
Case 1	55.4/F		Fever only	Group IV	37.4	C	*S. pyogenes*	Blood
Case 2	17/F		Fever only	Group IV	36.1	C	*S. pneumoniae*	Blood
Case 3	8.4/F	Sickle cell disease	Fever only	Group IV	39.9	NA	*E. coli*	Urine
Case 4	20.1/F	Traumatic brain injury	Fever only	Group IV	37.2	C	*E. coli*	Urine
Case 5	26.2/F	Reactive airway disease	Fever only	Group IV	39.7	NA	*E. coli*	Urine
Case 6	40.1/F	History of BPD	Fever only	Group IV	39.8	C	*P. aeruginosa*	Urine
Case 7	53/M	Cornelia De Lange	Fever only	Group IV	37.3	NA	*E. faecalis*	Urine
Case 8	6.1/M	History of BPD	Fever only	Group IV	31.2	C	*S. aureus*	Abscess
Case 9	57/M	TPN dependent, reactive airway disease	Fever only	Group IV	38.5	F	*S. aureus*	Abscess
Case 10	38.5/M	TPN dependent	URTI	Group IV	39.5	NA	*K. pneumoniae*	Blood
Case 11	13.7/F		URTI	Group IV	34.2	C	*S. aureus*	Abscess
Case 12	31.7/F		URTI	Group IV	35.9	C	*M. pneumoniae*	NP
Case 13	89.5/M		URTI	Group IV	38.9	F	*M. pneumoniae*	NP
Case 14	106.9/F	Cerebral palsy	LRTI	Group IV	39.0	C	*S. pneumoniae*	Blood
Case 15	27.4/F	Congenital heart disease	LRTI	Group IV	37.6	NA	*E. faecalis C. glabrata*	Blood
Case 16	199.1/M	Cerebral palsy	LRTI	Group IV	39.5	NA	*S. aureus*	Pleural fluid
Case 17	5.9/F		GI illness	Group IV	37.1	F	*B. pertussis*	NP
Case 18	11.8/M		Unclassified	Group IV	39.1	C	*B. pertussis*	NP
Case 19	3.9/M		URTI	Group II	33.1	C	*B. pertussis*	NP
Case 20	6.6/F	History of BPD	URTI	Group II	19.6	C	*B. pertussis*	NP
Case 21	66.3/F		LRTI	Group II	26.3	B	*M. pneumoniae*	NP
Case 22	34.1/M		LRTI	Group II	29.6	C	*S. pneumoniae*	Pleural fluid

Abbreviation: BPD, bronchopulmonary dysplasia; URTI, upper respiratory tract illness; LRTI, lower respiratory tract illness; GI, gastrointestinal; Ct, cycle threshold; NP, nasopharynx; NA, not available (Specimens were not able to be identified to species level using molecular testing)