Diagnostic monitoring of adenovirus infections in the immunocompromised host

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Human adenoviruses

Double-stranded DNA-Virus
Genome size: ~36 kb

7 species (subgenera): A-G

Species | Types (serotyping/computational analysis)
---|---
A | 12, 18, 31, 61
B | 3, 7, 11, 14, 16, 21, 34, 35, 50, 55, 66, 68
C | 1, 2, 5, 6, 57
D | 8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, 56, 58, 59, 60, 63, 64, 65, 67, 69-75
E | 4
F | 40, 41
G | 52

Most newly identified HAdV types resulted from homologous recombination events

## HAdV infections in immunosuppressed patients

### Organ-Tropism of HAdV Species

<table>
<thead>
<tr>
<th>Letter</th>
<th>Organ Tropism</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Intestinal tract; CNS</td>
</tr>
<tr>
<td>B</td>
<td>Respiratory tract; Eye; Intestinal tract; Genitourinary tract; CNS</td>
</tr>
<tr>
<td>C</td>
<td>Respiratory tract; Intestinal tract; Liver</td>
</tr>
<tr>
<td>D</td>
<td>Eye; Intestinal tract; CNS</td>
</tr>
<tr>
<td>E</td>
<td>Respiratory tract; Eye</td>
</tr>
<tr>
<td>F, G</td>
<td>Intestinal tract</td>
</tr>
</tbody>
</table>

### Clinical Manifestations

- Gastro-enteritis
- Pneumonia
- Hemorrhagic cystitis
- Meningoencephalitis
- Hepatitis
- Keratoconjunctivitis
- Respiratory infections
- Multiorgan failure (MOF)
Lethal invasive HAdV infections in pediatric allo-SCT

*Lion T et al. Leukemia 2010, 24(4):706-14*

138 patients

- All pts: n=138, 6%
- All deaths: n=36, 22%
- TRM: n=26, 31%
Adenoviremia and transplant-related mortality

Lion T, Blood 102(3): 114-20, 2003

HAdV positivity in PB ↔ TRM (p<0.001)

Quantification by RQ-PCR:
Rising virus load before onset of symptoms

Definition of critical HAdV threshold levels in PB for onset of treatment

Teramura T. BMT 2004, 33:87-92
4x10³ cp/ml

10⁴ cp/ml

> 10² cp/ml in high-risk patients
> 10³ cp/ml in intermediate-risk patients
> 10³ or > 10⁴ cp/ml in low-risk patients

Any HAdV species (subgenus) may occur
Disseminated disease: species C, A, B → Diagnostics
Viremia with multiple HAdV species
Switch of HAdV species during the posttransplant course
Documented efficacy of antivirals in HAdV infections

**Cidofovir**
- Currently primary anti-HAdV agent for pre-emptive therapy
- Efficacy against all HAdV species > gain time for T-cell recovery
- Low bioavailability and nephrotoxicity


**Brincidofovir**
- Safe, but limited data in immunocompromised patients

**Ribavirin**
- Documented in vitro activity against HAdV-C only
- Questionable therapeutic effect in vivo > added value against HAdV-C?

*(Morfin F. Antivir Ther 2009, 14:55-61; Lankester A. CID 2004, 38:1521-5); Abe S. BMT 2003, 32:1107-8*

**Ganciclovir**
- Modest activity due to inefficient phosphorylation (lack of TK in HAdV)


**Foscarnet**
- No activity

*(Naesens L. Antimicrob Agent Ther 2005, 49:1010-16)*
## Incidence of HAdV viremia in patients after HSCT

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Adults</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Viremia (lethal)</td>
<td>Viremia (lethal)</td>
</tr>
<tr>
<td>2013</td>
<td>Hiwarkar et al</td>
<td>12% (1/14)</td>
<td>15% (n.r.)</td>
</tr>
<tr>
<td>2012</td>
<td>Sive et al</td>
<td></td>
<td>16% (2/7)</td>
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<tr>
<td>2012</td>
<td>Taniguchi et al</td>
<td>9% (4/10)</td>
<td>15% (0/3)</td>
</tr>
<tr>
<td>2012</td>
<td>Watson et al</td>
<td></td>
<td>16% (2/7)</td>
</tr>
<tr>
<td>2011</td>
<td>Öhrmalm et al</td>
<td>3% (0/2)</td>
<td>15% (0/3)</td>
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<tr>
<td>2010</td>
<td>Lion et al</td>
<td></td>
<td>10% (8/16)</td>
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<tr>
<td>2009</td>
<td>de Pagter et al</td>
<td></td>
<td>31% (3/19)</td>
</tr>
<tr>
<td>2008</td>
<td>Gustafson et al</td>
<td>15% (2/4)</td>
<td>15% (1/2)</td>
</tr>
<tr>
<td>2007</td>
<td>Sivaprakasam et al</td>
<td></td>
<td>11% (3/8)</td>
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<tr>
<td>2007</td>
<td>Kalpoe et al</td>
<td>5% (1/5)</td>
<td>14% (3/8)</td>
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<tr>
<td>2006</td>
<td>Yusuf et al</td>
<td></td>
<td>21% (1/37)</td>
</tr>
<tr>
<td>2005</td>
<td>van Tol et al</td>
<td></td>
<td>6% (7/11)</td>
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<tr>
<td>2005</td>
<td>Walls et al</td>
<td></td>
<td>42% (2/7)</td>
</tr>
<tr>
<td>2004</td>
<td>Avivi et al</td>
<td>14% (3/3)</td>
<td></td>
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<tr>
<td>2002</td>
<td>Chakrabarti et al</td>
<td></td>
<td>3% (2/2)</td>
</tr>
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</table>
Preemptive treatment based on RQ-PCR screening in PB

Cidofovir (± Ribavirin) at first HAdV positivity in PB

Treatment for ≥ 2 weeks

Adoptive transfer of donor-derived HAdV-specific T cells

(Feuchtinger T et al., BJH 134: 64-76, 2006)

Isolation of PBMC from donor by density gradient centrifugation

Stimulation ex vivo with HAdV antigen C
10⁹-10⁹ PBMC in RPMI for 16 h at 37°C

Magnetic enrichment of IFN-g secreting T-cells
(Cytokine Secretion System® and CliniMACS® device-Miltenyi®)

Transfer of HAdV-reactive T-cells without further in vitro expansion
(1x10³-5x10⁴ T-cells/kg recipient body weight)
Preemptive treatment based on RQ-PCR screening in PB

Cidofovir (± Ribavirin) at first AdV positivity in PB
Treatment for ≥ 2 weeks

Adoptive transfer of donor-derived AdV-specific T cells
(Feuchtinger et al., BJH 134: 64-76, 2006)

Preliminary conclusion:
Success → early onset of treatment

Rational basis for earlier initiation of treatment?
Adenoviruses and allogeneic HSCT

HAdV-related disease

De novo infection

Reactivation from latent/persistent infection
Sites of adenovirus persistence

<table>
<thead>
<tr>
<th>Site</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary tract</td>
<td>Echavarria, JCM 1999, 37:686-9</td>
</tr>
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</table>

Other sites?
HAdV persistence in the gastrointestinal tract

Detection of HAdV in stool and viremia

Leukemia 2010, 24(4):706-14

Positivity in stool: 37%

- max. HAdV load ≤ 1x10^6/g
- no/slow kinetics

- max. HAdV load 5x10^6-10^{11}/g
- rapid kinetics

No viremia (p< 0.001) >70% viremia

Types A31, B03, C01, C02, C06 and F41

Ad species

Number of Ad positive biopsies
HAdV persistence in the GI tract

Kosulin K et al. Clin Microbiol Infect 2016, 22(4):381.e1-8

143 immunocompetent children: 576 GI biopsies

HAdV-positive biopsies
pos=63; neg=513

HAdV-positive patients
pos=44; neg=99

HAdV persistence vs reactivation post HSCT

Distribution of HAdV species

HAdV persistence in the GI tract ↔ risk of reactivation and disseminated disease?
Detection of HAdV in the GI tract by in situ hybridization


Hybridization pattern of persistent HAdV

Hybridization pattern of reactivated HAdV post HSCT

Presence of virus persistence in the GI tract: impact on preemptive HAdV treatment strategies in the future?
Switch of HAdV species during the posttransplant course

![Graph showing Patient B.F. HAdV Positivity in Stool](image1)

![Graph showing Patient B.F. HAdV Positivity in Peripheral Blood](image2)

6 weeks
Intestinal HAdV infection and viremia

*Lion T. Leukemia* 2010, 24(4):706-14

Monitoring of intestinal HAdV infection by RQ-PCR: basis for antiviral treatment

Intestinal infection

Viral load $> 1 \times 10^6 / g$ [confirmed by Jeulin H. Clin Microbiol Infect 2011]

Rapid proliferation kinetics

High risk of invasive infection

Preemptive treatment warranted
Screening by pan-HAdV PCR pre-transplant

Recipient: stool

Screening in stool 1x/week until day 28

- HAdV-negative w/o high-risk
  - RQ-PCR testing discontinued
  - RQ-PCR testing in stool 1x/∼2weeks
  - RQ-PCR testing in stool 1x/∼month

- HAdV-positive w/o high-risk
  - RQ-PCR testing in stool 1x/∼2weeks

- HAdV-positive + high-risk*
  - HAdV positive: load <10^6 copies w/o high risk
  - HAdV positive: rising load and/or load ≥ 10^6 copies/g
  - HAdV – negative: rising load and/or load ≥ 10^6 copies/g

- HAdV-positive + high-risk*
  - HAdV positive: load <10^6 copies/g + high risk

Donor: PB

Testing for presence of HAdV-specific T-cells

- Present

Testing for HAdV-specific T-cells in the recipient
- Reduction of immunosuppression, if possible
- Treatment by Cidofovir/Ribavirin***

Adoptive T-cell transfer

Isolation of HAdV-specific T-cells from the donor

- Poor response ****
Future treatment strategies

Introduction of novel antiviral drugs
- ganciclovir triphosphate
- lipid esters of cidofovir

Vaccination strategies
- lysate/peptide-loaded DCs

Improved adoptive T-cell transfer

Original stem cell donors
- Rapid in vitro expansion (< 2 weeks)

Stimulation by viral antigens
- Selection of IFNγ secreting virus-specific T cells

Donor registry
- Partially HLA-matched HAdV-specific cells from seropositive healthy donors

Third party donors

Healthier donors with common HLA polymorphisms

Virus-specific T-cell line bank

Multi-virus specific T-cells (CMV, HAdV, EBV, ...)

References:
Papadopoulou Sci Transl Med. 2014 25 242ra83
- Asaratnam JR, Leen AM Ann Transl Med 2015, 3:278-83
- Leen AM. Blood 2013, 121: 5113-23;
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Adoptive T-cell transfer

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