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Adenovirus infections in Bordeaux University Hospital 2008–2010: Clinical and virological features

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ABSTRACT

Background: Transversal epidemiological data on adenovirus infections in a hospital setting, including both immuno-competent and transplanted patients, are limited and rarely contain the application of molecular virology.
Objectives: To describe the clinical characteristics and molecular epidemiology of adenovirus infections in Bordeaux University Hospital from 2008 to 2010 (clinical data, viral load and adenovirus species distribution).
Study design: Adenovirus DNA quantification (qPCR) and typing (sequencing of hexon and protein VI genes and protein VI polymerase chain reaction (PCR) product analysis) were applied retrospectively to 215 clinical samples from 105 adenovirus-infected patients (2008–2010, Bordeaux University Hospital). Clinical data were recovered and analysed for 73 children and 25 adults.
Results: Viral loads were measured in stools, upper and lower respiratory fluids, blood, urine and digestive tract biopsies; the highest values were observed in stools and respiratory samples. Stool viral loads were comparable whatever the immune status. Adenovirus was typed in 57 patients: species *Human adenovirus (HAdV) C* dominated ($n = 36$), followed by B ($n = 15$), F ($n = 5$) and D ($n = 1$). We could demonstrate no association between HAdV species and load or clinical severity (observed in most patients). In the immuno-compromised, in contrast to immuno-competent patients, adenovirus infections presented no seasonal variation. Co-infections were frequent: mostly bacterial in immuno-competent children (33%) and viral in immuno-compromised people (34%).
Conclusions: The species HAdV *C* dominates the local ecology, in both respiratory and digestive tract infections, independently of the patient's immune status. Adenovirus infections, often associated with co-infection of bacterial or viral agents, frequently lead to severe clinical consequences in hospital patients.

1. Background

Adenoviruses (HAdVs) represent a common infectious aetiology in children under 5 years. Their efficient aerosolised and faecal–oral spread mostly causes self-limited, occasionally nosocomial, febrile respiratory illness and gastroenteritis.[1–4] Recently, HAdVs have also emerged as life-threatening opportunistic agents in severely immuno-suppressed patients, such as stem cell recipients,[5–10] leading to HAdV 'disease', affecting one or several organs.[5,9] Digestive infection often precedes viraemia,[11–14] which may lead in turn to disseminated disease, associated with high mortality,[5,9,15–17], in spite of cidofovir treatment.[18,19] The major risk factors have been identified as young age and immunologic impairment.[5,16,18] However, severe infections may also occur in immuno-competent patients.[17,20–22]

Abbreviations: HAdV, adenovirus; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV6, human herpes 6 virus; BKV, BK virus.

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Table 1
Patients clinical characteristics.

Patient type	Patient number (n = 98)	Samples (n = 215)	Clinical expression n (%)		Cidofovir treatment N (%)	Clinical severity n (%)
Immunocompetent children	45	54	Digestive Respiratory Fever Other Death	23 (51) 20 (44) 2 (9) 2 (9) 1 (2)	0	37 (79)
Children with underlying disease	19	21	Digestive Respiratory Fever Other Death	6 (32) 7 (37) 2 (11) 5 (26) 1 (5)	0	10 (50)
Children with hemopathy	9	42	Asymptomatic Cystitis Diarrhoea Death	1 1 1 0	2 (22)	2 (33)
Adults with underlying disease	3	6	Pneumopathy Colitis Fever Death	1 1 1 1	0	3 (100)
Adults with hemopathy	21	92	Asymptomatic Digestive Respiratory Death	16 (76) 5 (24) 1 (5) 3 (14)	6 (29)	18 (82)

The five groups of patients are identified, together with the corresponding number of samples, the dominant clinical features, cidofovir prescription and the existence of at least one of the previously established severity criteria.

Paediatric HAdV infections are often diagnosed in an epidemic context.[1,9] However, viral reactivation from a yet poorly identified lymphoid reservoir may commonly occur in immuno-suppressed patients.[9,11,23] Presently, diagnosis mostly relies on HAdV DNA detection by qPCR in peripheral blood,[5,9,12,16,24,25] which could also have a prognostic value.[10,19,26] Other biological fluids or biopsies are tested, depending on the symptoms.[5] Early HAdV stool load monitoring has also been proposed.[11]

HAdVs display an important genetic variability,[5] which drives their assignment to species (A–G) and types (1–53).[27,28] This diversity could have functional consequences on clinical severity and outcome of HAdV infections[9,29,30]; HAdV-1, -2 and -5, belonging to species *HAdV C*, were described as frequently associated with disseminated disease in highly immuno-suppressed patients.[5,10] However, HAdV typing is not commonly monitored in clinical virology laboratories.

Co-infections do occur frequently, both in immuno-competent[1] and immuno-compromised people[12,24,31]; they involve bacteria, parasites, fungi or representatives of other viral families, such as the polyomavirus BK virus (BKV) and various Herpesviruses (cytomegalovirus (CMV), Epstein–Barr virus (EBV) and human herpes 6 virus (HHV6)). The existence of inter-pathogen interactions has been suggested.[12]

2. Objectives

Epidemiological data on HAdV infections using modern virological diagnosis tools are still limited and currently absent in our region. To address this lack of knowledge, here we describe the molecular epidemiology and clinical characteristics of HAdV infections in Bordeaux University Hospital, from 2008 to 2010. We have measured viral load in various biological samples and characterised HAdV species distribution in immune-competent and immune-compromised patients.

Using this data set, we investigated a possible association between the severity of clinical manifestations and the HAdV species or viral load. Furthermore, we carefully identified clinically significant co-infections.

3. Study design

3.1. Samples

All samples used in this study were initially tested positive for HAdV during the 2008–2010 period in the routine diagnostics of the Bordeaux University Hospital using an in-house qualitative polymerase chain reaction (PCR) assay targeting hexon (B Lina, personal communication) and stored at −80 °C until further use.

3.2. HAdV DNA quantification

For the re-evaluation in 2011, DNA extraction from the initial positive samples was performed with Roche Magnapure Compact instrument (Roche total nucleic acids reagents), and HAdV quantification was obtained using a different pair of qPCR primers targeting hexon.[32,33] Using this method, our laboratory passed the quality control for molecular diagnostics (QCMD) HAdV 2011 panel. Results were expressed as either DNA copies/ml (in whole blood, bronchoalveolar lavage fluids and urine), DNA copies/g stools or DNA copies μg^{-1} DNA (respiratory swabs, upper respiratory secretions and biopsies). Under our extraction conditions, linear quantification could be achieved approximately between 500 and 10^{10} copies/ml (or /g stools, or /μg DNA). Therefore, weakly positive and strongly positive results were, respectively, expressed as <500 and >10^{10}.

3.3. HAdV typing

Three complementary methods were used.

Nucleotidic sequencing of the hexon Hyper Variable Region-7 fragment using ABI 3500 automatic sequencer was performed, allowing precise genotyping.[34] Confirmatory or additional sequences were obtained by amplifying a region of the HAdV