ORIGINAL ARTICLE VIROLOGY

Adenovirus infection and disease in paediatric haematopoietic stem cell transplant patients: clues for antiviral pre-emptive treatment

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Abstract

Human adenovirus (HAdV) infections constitute a major cause of morbidity in paediatric haematopoietic stem cell transplant (HSCT) patients. New antiviral treatments offer promising perspectives. However, it remains challenging to identify patients at risk for disseminated infection, and who should receive early antiviral intervention. We conducted a longitudinal study of allogeneic HSCT recipients, including weekly HAdV monitoring, to determine the risks factors associated with HAdV infection and dissemination, and to assess whether HAdV loads in stools may be used as surrogate markers for HAdV dissemination. Between September 2010 and December 2011, out of 72 patients, the cumulative incidence rates at day 100 of HAdV digestive infection, systemic infection and related disease were 35.9%, 24.0%, and 18.3%, respectively. In multivariate analysis, the risk factors for HAdV digestive and systemic infection were cord blood and *in vitro* T-cell depletion. Graft-versus-host disease (GVHD) grade >2 was also associated with systemic infection. In patients with HAdV digestive shedding, GVHD grade >2 and HAdV load in stools were the only risk factors for systemic infection. The median peak levels of HAdV in stool were 7.9 and 4.0 $\log_{10}$ copies/mL, respectively, in patients with HAdV systemic infection and in those without. HAdV monitoring in stools of paediatric HSCT recipients receiving cord blood or *in vitro* T-cell depleted transplants helps to predict patients at risk for HAdV systemic infection. Our results provide a rationale for randomized controlled trials to evaluate the benefit of anti-HAdV pre-emptive treatments based on HAdV DNA levels in stools.

Keywords: Adenovirus, adenovirus disease, adenovirus infection, antiviral treatment, dissemination, haematopoietic stem cell transplant, plasma, stool

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Introduction

Viral diseases constitute a major cause of morbidity and mortality after haematopoietic stem cell transplantation. Whereas reactivations of *Herpesviridae* family viruses are usually well controlled through effective monitoring and antiviral treatments, human adenovirus (HAdV) infections remain associated with high morbidity in paediatric haematopoietic stem cell transplant (HSCT) patients [1].

Although HAdVs do not produce lifelong infections, they may persist and lead to endogenous reactivations, especially from the digestive tract in immunocompromised individuals [2–4]. Paediatric HSCT recipients are more susceptible to adenovirus infection than are adults. The incidence of HAdV

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TABLE 1. Patient characteristics, transplantation modalities, and outcomes

Parameters	Value	*N*	Median (IQR) or %
Age at SCT (years)		72	6.5 (3.8–11.6)
Gender	Male	42	58.3
	Female	30	41.7
Diagnosis	AML	9	12.5
	ALL	21	29.2
	JMML	9	12.5
	Lymphoma	5	6.9
	Haemoglobinopathy	11	15.3
	Others	17	23.6
TBI	Yes	22	35.5
Allograft in CR1	Yes	45	62.5
Stem cell source	Peripheral blood	5	7.0
	Bone marrow	53	73.6
	Cord blood	14	19.4
Donor type	Geno-identical	24	33.3
	Haplo-identical	5	6.9
	≥9/10 HLA-unrelated	29	40.3
	≥4/6 cord blood	14	19.4
Acute GVHD	No	31	43.1
	Grade ≤2	10	13.9
	Grade >2	25	34.7
	Prior death	6	8.3
HAdV digestive infection	Yes	28	38.9
HAdV systemic infection	Yes	18	25.0
HAdV probable disease	Yes	13	18.1
HAdV disease symptoms	Fever	10	76.9
	Diarrhoea	10	76.9
	Respiratory disease	7	53.9
	Hepatitis	11	84.6
	Encephalitis	3	23.1
	Multivisceral failure	1	7.7
	Pancreatitis	1	7.7
	Haemorrhagic cystitis	2	15.4
Death	M3	7	9.7
	M12	13	16.7

ALL, acute lymphoid leukaemia; AML, acute myeloid leukaemia; GVHD, graft-versus-host disease; HAdV, human adenovirus; HLA, Human Leucocyte Antigen; IQR, interquartile range; JMML, juvenile myelomonocytic leukaemia; M3, 3 months after graft; M12, 12 months after graft; SCT, stem cell transplantation; TBI, total body irradiation.

viraemia has been reported to be 6–28% in children, and only up to 6% in adults [3–7].

Treatment options are limited [8]. Cidofovir and ribavirin are antiviral drugs that are effective *in vitro*, although their use has not been validated with randomized trials [4,8,9]. Some new strategies under clinical investigation have been raising new hopes. First, CMX001 (brincidofovir), a lipophilic conjugate of cidofovir, providing a larger tissue distribution and a higher intracellular concentration than cidofovir, has shown encouraging results in a few case reports [10,11]. Second, the infusion of anti-HAdV T-cells expanded from the donor or from a third party has been reported to be an effective strategy, provided that the treatment can be initiated quickly to enable control of viral replication [12,13]. Because early interventions are key to antiviral efficacy [8,13], there is a need, first, to identify patients with a high risk of HAdV disseminated infection, and second to define when to initiate pre-emptive or curative treatment.

In this study, we assessed the risk factors associated with HAdV digestive shedding, blood infection and HAdV disease in a single paediatric HSCT centre, and determined whether HAdV loads in stools and plasma may be used as surrogate markers for HAdV dissemination. In agreement with previous reports [3,14], the risk factors that we have identified will make it possible to target patients requiring a narrow follow-up, and the HAdV load levels in stools that we have defined may be used to trigger therapeutic intervention.

Patients and methods

Patients

Between September 2010 and December 2011, 72 children (30 females and 42 males) who underwent allogeneic stem cell transplantation at Robert Debré Hospital (Paris, France) were prospectively followed for up to 12 months. Patient characteristics and modalities of transplantation are summarized in Table 1. Details of medical history and clinical follow-up are given in Doc. S1.

Patients with probable HAdV disease were treated with cidofovir. If renal function was normal, cidofovir was administered at 5 mg/kg once weekly with probenecid. If renal function was impaired (creatinine level twice the normal level), cidofovir was administered at 1 mg/kg three times weekly with probenecid. Ribavirin or brincidofovir was given to patients not responding to cidofovir. Brincidofovir was used only when it was available for compassionate use. When possible, immunosuppressive therapy was tapered. Two patients received specific anti-HAdV cytotoxic lymphocytes from either a haploidentical related donor or a third-party donor. Cidofovir was given to some patients who had persistent high HAdV DNA levels in stools and were considered to be at high risk for disseminated infection.

Ethics statement

The study was carried out in accordance with the Declaration of Helsinki. This study was a non-interventional study with no additional procedures. Biological material and clinical data were obtained only for standard viral diagnostic according to physicians' prescriptions. Data analyses were carried out with an anonymous database. According to the French Health Public Law (CSP Art L 1121-1.1), such a protocol is exempt from informed consent application. The two parents or guardians of these paediatric patients gave written informed consent to all aspects of the transplantation procedure and to the use of medical records for research.

Virus monitoring

Whole blood samples were collected weekly and tested for cytomegalovirus (CMV) and Epstein–Barr virus by quantitative PCR. HAdV infections were monitored on a weekly basis by quantitative PCR in plasma and stool samples until discharge or

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