Quantification of Adenovirus DNA in Plasma for Management of Infection in Stem Cell Graft Recipients

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We used a real-time polymerase chain reaction method for quantification of adenovirus to monitor the dynamics of viral DNA load in plasma in pediatric stem-cell graft recipients. Two cases are described to demonstrate that detection and quantification of the adenovirus DNA load at regular intervals may be important to document the stage of adenovirus infection, to make decisions on clinical intervention, and to accurately monitor the response to antiviral therapy.

Adenovirus (AdV) infection is a frequent complication in children who receive an allogeneic stem cell transplant (SCT) or a solid-organ transplant. In allogeneic SCT recipients, AdV infections are predominantly found in those who have received a graft from a matched unrelated donor or a human leukocyte antigen–mismatched family donor. Infections in this group of patients lead to disseminated disease at the relatively high rate of ≥50% and are often fatal ([1–3]; unpublished data).

The precise mechanism of AdV infection or reactivation remains to be demonstrated, but it is most probably related to the presence of infectious AdV during a prolonged period of inadequate immune recovery. In immunocompromised hosts, the initial, nondisseminated phase of the infection is often asymptomatic. In a subsequent stage, more obvious clinical manifestations may appear: for example, enteritis and hemorrhagic cystitis. Finally, progression to fulminant disseminated disease manifests as pneumonia, hepatitis, encephalitis, and multiple-organ failure, and it has a fatal outcome in most cases.

At present, 51 AdV serotypes have been identified, which are classified into 6 subgroups (A–F). In pediatric immunocompromised patients, AdV subgroups A–C are most frequently isolated in connection with clinical disease [4]. AdV infection is diagnosed by isolation of the virus from presumed sites of initial infection and reactivation—that is, from stool and urine samples and throat swabs—by use of conventional culture systems. AdV can also be cultured from blood samples or detected by PCR in specimens of various tissues [5, 6]. There is evidence of an association between the simultaneous isolation of AdV from multiple sites and the occurrence of clinical disease [1, 3, 7]. However, insight into the biology and dynamics of AdV infections is still limited.

Until now, management of AdV infection has been a major challenge. Several treatment modalities have been reported in the literature, including administration of intravenous immunoglobulins (IVIGs), administration of antiviral agents such as ribavirin and cidofovir, and infusion of lymphocytes from a donor [8–11]. Both beneficial effects and failures of these therapeutic interventions were reported in those studies. A major obstacle to drawing conclusions about the efficacy of the different treatment regimens is the heterogeneity of the investigated populations of infected individuals, especially with regard to the duration and stage of the AdV infection at the initiation of treatment. A limited number of tools are currently available to accurately monitor disease activity and to draw conclusions about the response to therapeutic intervention.

It has been reported that quantification of viral DNA load in leukocytes or plasma is a valuable tool to monitor cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections in immunocompromised hosts [12–14]. Use of real-time quantitative PCR technology (RQ-PCR) allows accurate early detection and follow-up to detect possible progression or dissemination of the disease, as well as monitoring of the therapeutic response. Recently, we have reported on the value of using qualitative PCR to detect AdV for the monitoring of AdV infections [15]. On the basis of this “pan-AdV” PCR method, an RQ-PCR assay has been developed that permits quantification of AdV load in individuals with PCR results positive for AdV. The RQ-PCR assay was first developed for subgroup C viruses, because infection with this subgroup is predominant in our patients. Further evaluation of this assay will be described elsewhere (unpublished data). In brief, we extracted DNA from plasma samples by standard procedures and amplified 10 μL of DNA-containing extract using RQ-PCR and the oligonucleotide primers described by Echavarria et al. [16]. On the basis of the described probe sequence, a molecular beacon was made that...
was labeled with 5’ Texas Red and 3’ DABSYL (Eurogentec). As a standard for quantification, we used DNA isolated from a dilution series of a purified stock of adenovirus type 5 that had been titrated by use of HPLC [17]. Threshold cycle values were obtained with this dilution series by means of RQ-PCR and the iCycler IQ system (BioRad Laboratories). These values were used to construct a standard curve and to determine the viral DNA load in the plasma samples. In the present report, 2 cases are presented that illustrate the potential relevance of AdV DNA load quantification for monitoring the dynamics of AdV infection and evaluating the response to therapeutic interventions.

**Patient 1.** A 10-year-old boy with myelodysplastic syndrome (MDS) was treated with a myeloablative conditioning regimen (busulphan [16 mg/kg], melphalan [140 mg/m²], cyclophosphamide [200 mg/kg], and antithymocyte globulins [10 mg/kg]). Subsequently, he received a CD34⁺ cell–enriched peripheral blood SCT (PSCT) obtained from 1 of his haploidentical parents. Nonengraftment was diagnosed at day 28 after the procedure. Fourteen days later, the patient received a second CD34⁺ cell–enriched PSCT obtained from the other parent. During follow-up, only minimal engraftment was seen. In accordance with our standard procedures, IVIGs were administered (150 mg/kg per week) starting at approximately day 21 after the first procedure. Neither pharmacological graft-versus-host disease prophylaxis nor antiviral prophylaxis was administered. Routine screening for AdV infection was performed by means of conventional cultures of stool and urine samples and throat swabs and by PCR analysis of plasma samples.

Routine screening for AdV resulted in isolation of AdV type 2 (group C) from a stool sample on day 27 after the first PSCT procedure (figure 1A). During the subsequent period, AdV type 2 persisted in the intestinal tract. AdV had not been simultaneously isolated from other sites on any occasion. Of note, up to day 21 after the second PSCT procedure, symptoms possibly related to AdV infection, including enteritis, were definitely absent. On day 6 after the second PSCT procedure, AdV was detected for the first time in plasma by means of PCR, at a level of 9000 viral particles/mL. Because of an additional 100-fold increase of viral DNA load in plasma, preemptive treatment with cidofovir (5 mg/kg per week) was initiated on day 17 after the first PSCT procedure. On day 21, the patient presented with clinical symptoms that might have been due to AdV infection: fever (temperature, >38°C) and an increase in the levels of transaminases and bilirubin. As clinical symptoms—that is, hepatitis—progressed and the virus load further increased, ribavirin (loading dose, 30 mg/kg; maintenance dosage, 60 mg/kg per day) was added to the antiviral treatment regimen on day 24 after the second PSCT procedure. Despite receipt of this treatment, a high virus load persisted. The patient’s clinical condition deteriorated, and he finally died on day 36. Apart from the fulminating AdV disease, no other cause of death could be identified.

**Patient 2.** A 6-year-old boy with MDS was treated with a myeloablative conditioning regimen (busulphan [16 mg/kg], melphalan [140 mg/m²], cyclophosphamide [200 mg/kg], and anti-thymocyte globulins [10 mg/kg]). He received a CD34⁺ cell–enriched PSCT from his haploidentical mother. Engraftment was confirmed on day 28. Still, immune reconstitution in this patient was extremely weak during the entire observation period: the CD3⁺ lymphocyte count remained <50 lymphocytes/μL, and cultures of whole blood samples revealed no lymphocyte proliferative responses. IVIGs were administered (150 mg/kg per week) starting on approximately day 21. Neither pharmacological graft-versus-host disease prophylaxis nor antiviral prophylaxis was administered. Routine screening for AdV infection was performed as described for patient 1. AdV type 31 (group A) was isolated from a stool sample on day 30 (figure 1B). Further analysis of this AdV type 31 isolate by use of sequence analysis revealed 1 nucleotide mismatch with the probe. RQ-PCR performed on a sample of the viral DNA yielded a positive result, which indicated that the virus, if present, would be detected in plasma as well. At follow-up, AdV was simultaneously isolated from urine samples and throat swabs. During a 4-week period during which cultures of samples from multiple sites were positive for AdV, probable AdV-related clinical symptoms were present in the form of (mild) diarrhea. From day 77 onward, AdV was not isolated from any site. In contrast to findings for patient 1, AdV was not detected in plasma by the “pan-AdV” PCR at any time point, which indicated that significant viremia was absent. Of note, no anti-AdV therapy was administered during the entire observation period. As of February 2002, the patient is alive and well.

**Discussion.** Quantitative monitoring of the viral DNA load is a powerful tool to understand the dynamics of viral infections, to support the decision to initiate antiviral treatment, and to evaluate the effect of this treatment. For example, studies on EBV reactivation in immunocompromised hosts have shown that an increase in virus load (as measured in samples of whole blood, leukocytes, or plasma) precedes the onset of signs and symptoms of disease. Moreover, preemptive treatment strategies have been developed on the basis of these data [12–14].

Management of AdV infections is still a difficult matter. Patients with localized infections (e.g., isolated intestinal infection) may require different treatment approaches than do patients who are prone to dissemination of the virus and progression to severe disease. Analogous to what has been shown for CMV and EBV infections, quantification of AdV DNA load appears to be an important aid to clinical care and decision making, as illustrated by the cases presented here. In the patients we describe, the difference in severity and outcome of the AdV infection was clearly reflected in the measured levels of AdV DNA in plasma.

For patient 1, a progressive increase in AdV DNA load in plasma was demonstrated, despite the absence of any clinical
indications of AdV-related disease. Antiviral therapy was initiated solely on the basis of the repeated positive stool culture results and the increase in the AdV DNA load. The apparent failure of therapy should be judged in light of the high virus load at initiation of therapy. The rapid increase of the virus load in the plasma of this patient points to the necessity for frequent screening of plasma samples in patients at risk for AdV infection or reactivation.

For patient 2, although AdV-related symptoms (i.e., enteritis) were present and AdV was isolated from multiple sites, AdV DNA could not be detected at any time in plasma. Of interest, AdV was cleared without administration of anti-AdV therapy and in the absence of demonstrable immune recovery. In this patient, initiation of any antiviral treatment might have led to an erroneous overestimation of its therapeutic potential. A number of studies have reported the possible benefits and the failure of AdV-directed treatment regimens [8–11]. On the basis of the results from our patients, we consider use of RQ-PCR to detect and quantify virus load to be an important tool for deciding whether and when to start treatment with antiviral therapy. In addition, we think that this method may be useful in future studies to evaluate the efficacy of antiviral treatment modalities. Therefore, we have recently included PCR-based detection and quantification of AdV in plasma in a prospective

Figure 1. Clinical and laboratory data for patient 1 (A) and patient 2 (B). Horizontal black lines, Duration of clinical symptoms and therapy; ○, positive adenovirus (AdV) culture results; ●, negative AdV culture results; blackened polygons, DNA load. Vertical black line at day 42 in A indicates the time of the second stem cell transplantation procedure (SCT). IVIGs, intravenous immunoglobulins.
study of pediatric allogeneic SCT recipients in order to accurately monitor AdV infections and the efficacy of various anti-viral treatment modalities.

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References