Incidence and Clinical Manifestations of Adenoviral Infection among Children Undergoing Allogeneic Stem Cell Transplantation

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ABSTRACT: **Background:** Adenoviral infection in children undergoing stem cell transplantation is associated with significant morbidity and mortality. Identification of adenoviral infection by polymerase chain reaction from blood facilitates accurate and rapid diagnosis and surveillance. The incidence of adenoviral infection among children undergoing SCT in Israel is not known.

Objective: To estimate the incidence of adenoviral infection in pediatric SCT patients and to characterize the morbidity associated with proven infection.

Methods: Blood samples obtained weekly from children who underwent allogeneic SCT were retrospectively tested for adenovirus using standard PCR. A total of 657 samples collected from 32 patients were examined. Correlation was made between the presence of adenovirus in samples and clinical records.

Results: Of the 32 patients 4 had adenoviral infection by PCR (12.5%). Clinical disease was present in all four patients concurrent with positive PCR. Gastrointestinal complaints and abnormal hepatocellular enzymes were uniformly present. One patient died due to disseminated disease. T cell depletion was a significant risk factor for adenoviral infection ($P = 0.03$).

Conclusions: In the patient population studied, the incidence of adenoviral infection in children undergoing SCT was 12.5%. The combination of gastrointestinal symptoms and abnormal hepatocellular enzymes should raise the suspicion of adenoviral infection, especially when occurring during the first few months after SCT.

IMAJ 2009; 11: 744–748

KEY WORDS: adenovirus, polymerase chain reaction, stem cell transplantation, immunosuppression, T cell depletion

Stem cell transplantation is a standard treatment for a variety of disorders including hematologic malignancies, solid tumors, congenital immune deficiencies, hemoglobinopathies and autoimmune diseases. Adenovirus has been increasingly recognized as a significant pathogen in SCT patients [1,2]. Children undergoing SCT are at greater risk of acquiring adenovirus infection than adults [3,4], with up to 42% having polymerase chain reaction evidence of adenoviral infection in the post-transplantation period [5]. In addition, moderate to severe graft-versus-host disease and pre-transplant T cell depletion are independent risk factors for development of adenoviral disease [6,7]. The clinical manifestations of adenovirus infection in SCT patients are diverse and include asymptomatic infection, localized disease (such as pneumonia, enterocolitis, hemorrhagic cystitis, hepatitis), as well as disseminated infection which may be rapidly fatal [8-11].

Following SCT, immunological responses to various infectious agents may be poor; therefore, serological diagnosis is not always useful. Molecular identification of adenoviral infection by PCR from blood and other readily available tissue facilitates accurate and rapid diagnosis and can be used for surveillance as well. The incidence and clinical manifestations of adenoviral infection among children undergoing SCT in Israel is not known. The aim of the present study was to estimate the incidence of adenovirus infection in blood samples of pediatric patients who underwent allogeneic SCT and to clinically describe those patients with proven infection.

PATIENTS AND METHODS

Blood samples obtained from 32 children who underwent allogeneic SCT at Rambam Medical Center between December 2002 and July 2006 were retrospectively tested. The group comprised 16 boys and 16 girls aged 2 months to 18 years (mean 7.4 years). Twelve underwent SCT due to malignancy (acute lymphoblastic leukemia in 8 and acute myeloid leukemia in 4). Other diagnoses included a variety of hereditary, immunological and hematological conditions such as thalassemia, myelodysplastic syndromes, Hurler syndrome, famil-

SCT = stem cell transplantation
PCR = polymerase chain reaction

ial hemophagocytosis, severe combined immunodeficiency, chronic granulomatous disease, etc. Of the 32 children, 25 received an allogeneic transplantation from a related donor, 3 had haploidentical transplants, and 4 received bone marrow from a matched unrelated donor. Thirteen patients were prepared for SCT by T cell depletion of varying degrees (partial and complete, 10 and 3 respectively).

Whole blood samples, stored at -70°C in the virology laboratory, were retrospectively tested for adenoviral DNA by PCR. These samples were collected weekly from each pediatric SCT patient during the 6 months following transplantation for routine cytomegalovirus screening. Altogether, 657 samples collected from 32 patients were available for testing. The mean number of samples per child was 20.5. Clinical and laboratory parameters were reviewed for all patients with positive PCR for adenovirus. This study was approved by the hospital Institutional Review Board.

IDENTIFICATION OF ADENOVIRUS DNA

DNA from patients' whole blood samples was extracted using the QIAamp DNA blood mini-kit according to the manufacturer's instructions (QIAgen Ltd., Crawly, England UK). For qualitative results, 5 µl of the DNA is amplified by conventional PCR, according to Xu et al. [12]. The group-specific primers are complementary to the a region of the hexon gene, conserved among all human adenovirus serotypes:

Forward primer: 5'-TTC-CCC-ATG-GCI-CAY-AAC-AC-3'

Reverse primer: 5'-CCC-TGG-TAK-CCR-ATR-TTG-TA-3'

PCR reaction was performed in 25 µl reaction volume containing 10 mM of each deoxynucleotide triphosphate, 0.2 mM of each primer, 1 U of Taq DNA polymerase and 5 µl of extracted DNA. Amplification was performed by 30 cycles of denaturation at 94°C for 60 seconds, annealing at 54°C for 45 sec, and elongation at 72°C for 120 sec. PCR products were detected by electrophoresis in 2% agarose gel stained with ethidium bromide. Each sample that tested positive was retested by the same method. Only those patients who had positive results on both occasions were defined as having adenoviral infection.

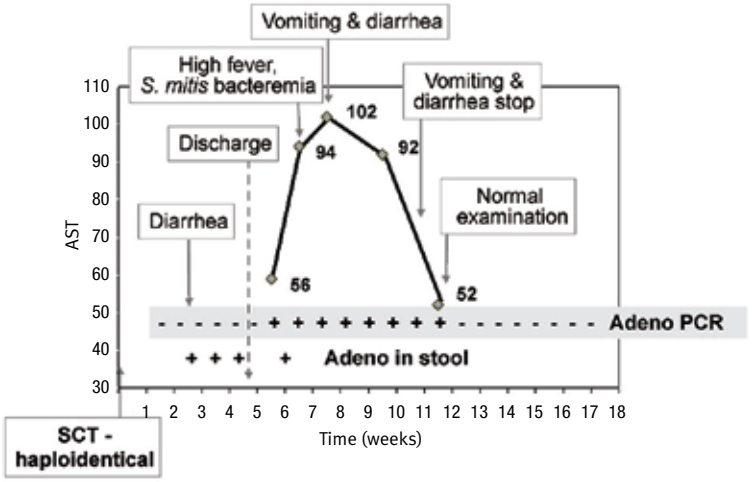
STATISTICAL ANALYSIS

Rates of adenoviral infection were compared between groups using the Fisher exact test, and $P \leq 0.05$ was considered significant.

RESULTS

Adenovirus infection was detected in 4 of the 32 patients (12.5%). Three of the four became ill within 3 months of SCT. Clinical disease was present in all four patients concurrent with positive PCR. Gastrointestinal complaints (usually diarrhea) and hepatocellular dysfunction (abnormal alanine and aspartate aminotransferase levels) were present in all cases.

Figure 1. Clinical and laboratory data during the 18 weeks following SCT in an 18 month old child with Wiskott-Aldrich syndrome (patient #1)



PATIENT 1 [FIGURE 1]

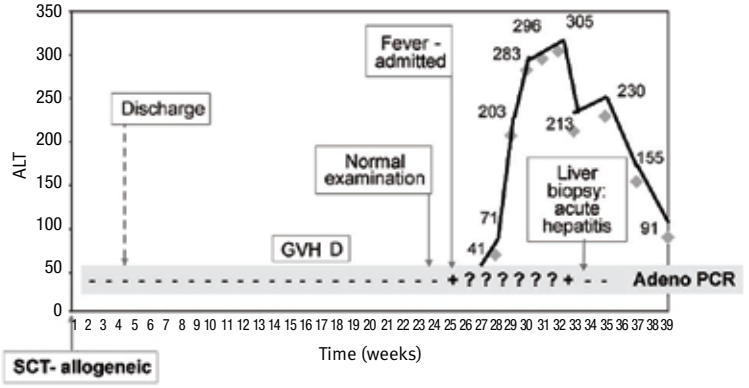
An 18 month old child with Wiskott-Aldrich syndrome underwent haploidentical SCT from his father due to severe autoimmune hemolytic anemia and refractory thrombocytopenia. T cell depletion was performed by positive selection of CD34 cells by immunomagnetic beads (Miltenyi, Biotech, Bergisch Gladbach, Germany). Two weeks after transplantation, while neutropenic, he developed intractable diarrhea and his stools were repeatedly positive for adenoviral antigen (at this time, PCR of blood was repeatedly negative). Engraftment occurred on day 19 post-transplantation and the patient was discharged 5 weeks after transplant only to be readmitted one week later due to high fever followed by vomiting, diarrhea and abnormal hepatocellular enzymes. Adenoviral PCR in blood became positive concurrently and persisted for almost 8 weeks. Adenoviral DNA disappeared from blood samples along with the resolution of clinical symptoms.

PATIENT 2 [FIGURE 2]

A 15 year old boy with T cell acute lymphoblasic leukemia underwent allogeneic SCT from his matched brother without T cell depletion. His post-transplant course was uneventful and he was discharged 4 weeks after transplantation. He developed acute graft-versus-host disease and later chronic GVHD, and was treated with immunosuppressive drugs. Six months after transplantation, during tapering of immunosuppressive therapy, he was admitted with fever and a rapid increase in serum AST and ALT. PCR for cytomegalovirus was negative. Due to the persistent elevation in serum

GVHD = graft-versus-host disease
AST = aspartate aminotransferase
ALT = alanine aminotransferase

Figure 2. Clinical and laboratory data during the 40 weeks following SCT in a 15 year old child with acute lymphoblastic leukemia (patient # 2)



hepatocellular enzymes and suspected GVHD, a liver biopsy was performed and non-specific hepatitis without evidence of cytomegalovirus infection or local GVHD was seen. At this time PCR of blood showed evidence of adenoviral infection with onset concurrent with the increase in hepatocellular enzymes. Unfortunately, some samples were missing, but 7 weeks after the first positive PCR another sample was positive, suggesting that the infection lasted throughout this period. After viral DNA disappeared from blood, the patient's symptoms slowly subsided, with liver enzymes returning to normal.

PATIENT 3 [FIGURE 3]

A 3 year old child with acute lymphoblasic leukemia and poor response to prednisone underwent allogeneic SCT from his matched sister. No T cell depletion was done. Engraftment of neutrophils occurred on day 8 post-transplant. Five weeks after transplantation he began to suffer from frequent vomiting, fever and headache. Lumbar puncture revealed pleocytosis with lymphocytic predominance and elevated cerebrospinal fluid protein. PCR for herpes simplex virus and enterovirus were negative, as were blood and CSF cultures. He was treated with broad-spectrum antibiotics. Examination of blood samples showed that adenoviral DNA was present almost 2 weeks before the onset of clinical symptoms. Adenoviral DNA disappeared from blood samples after 2 weeks. Six months after transplantation, during a routine outpatient visit, a mild upper respiratory infection was noted. During the following weeks he developed persistent diarrhea, vomiting and mild elevation of hepatocellular enzymes. PCR for adenovirus was positive shortly before the gastrointestinal symptoms appeared and became negative concurrently with the spontaneous resolution of symptoms.

PATIENT 4 [FIGURE 4]

A 2 year old child with severe combined immunodeficiency (bare lymphocyte syndrome) underwent a second haploidentical SCT from his mother due to non-engraftment of a previous haploidentical SCT from his father. The second transplantation was done 4 weeks after the first one. T cell depletion using positive selection of CD34 cells (Miltenyi) was done for both SCTs. The patient was severely neutropenic for 42 days. Four weeks after the second transplantation he developed high fever with a rapid elevation of liver enzymes. Graft rejection was suspected and he was treated with high dose steroids. Fulminant hepatitis rapidly ensued, followed by multiorgan failure and

CSF = cerebrospinal fluid

Figure 3. Clinical and laboratory data in the 29 weeks following SCT in a 3 year old child with acute lymphoblastic leukemia (patient # 3)

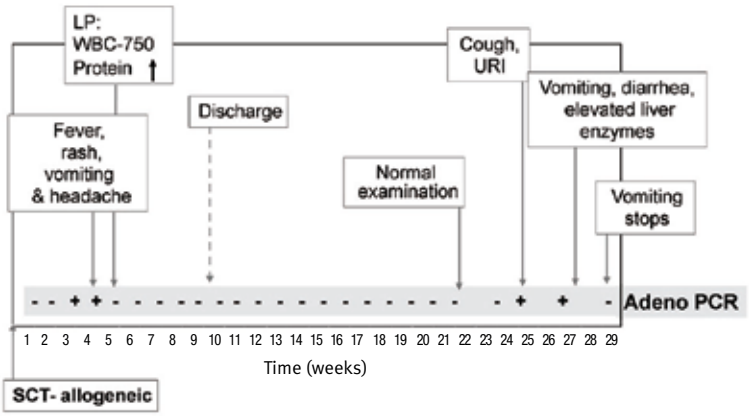
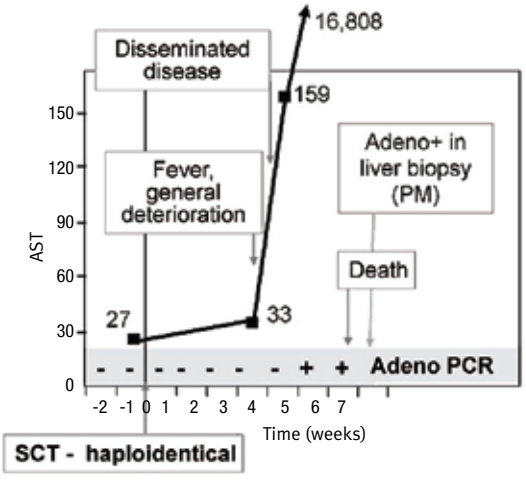


Figure 4. Clinical and laboratory data in the 7 weeks following SCT in a 2 year old child with severe combined immunodeficiency who underwent a second haploindentical SCT (previous haploidentical SCT 6 weeks prior; never engrafted) (patient # 4)



death. Examination of blood samples showed adenoviral DNA concurrent with his fulminant hepatocellular dysfunction. Postmortem liver biopsy showed adenoviral hepatitis. Blood and liver samples were negative for cytomegalovirus. Blood cultures were repeatedly negative except for one taken a few hours before death with growth of *Oligella ureolytica*.

T cell depletion used in haploidentical SCT was the only significant risk factor for adenoviral infection ($P = 0.03$), with two of three patients undergoing haploidentical SCT with T cell depletion developing adenoviral infection.

DISCUSSION

In the population studied, the incidence of adenoviral infection in children undergoing allogeneic SCT is 12.5%. These data are consistent with several reports that demonstrated adenoviral disease in 3–42% of pediatric SCT patients [1-5]. The broad spectrum of incidence is probably related to the use of different diagnostic tools as well as to population differences. Until recently, the diagnosis of adenoviral infection was based on serological tests that may be insensitive in heavily immune suppressed patients; on culture of clinical samples, which is time consuming and relatively insensitive; and by pathological examination, often available only post-factum.

The significant morbidity and mortality of adenoviral infection in SCT patients has favored the development of rapid and reliable methods for the detection of this virus, preferably before clinical manifestations and tissue damage evolve. The development of sensitive and specific molecular methods such as PCR can be considered a breakthrough in this regard [5,11,13,14]. Lion et al. [11] tested more than 5000 clinical samples including respiratory, blood, urine and stool that were obtained from 132 children who underwent SCT. Of these, 27% tested positive for adenovirus by PCR. Identification of adenoviral DNA in blood correlated significantly with clinical symptoms and with a very high mortality rate (82%). Among patients who developed disseminated infection, adenovirus was detected by PCR almost 3 weeks before the onset of clinical manifestations [11]. Other studies also showed that presumptive adenoviral viremia manifested as positive blood PCR could be an indicator of impending multiorgan infection or disseminated disease [13-16], thus opening a window of opportunity for possible intervention before the onset of full-blown disseminated disease.

In this study, all patients with positive PCR for adenovirus had clinical symptoms which, in retrospect, are consistent with adenoviral infection, appearing concurrently or shortly (1–2 weeks) after PCR becoming positive for adenovirus. Interestingly, all patients with positive adenoviral PCR had mainly gastrointestinal symptoms with hepatic involvement ranging from mild elevation of hepatocellular enzymes to fulminant hepatic failure. Several studies have demonstrated that diarrhea is the most common clinical presentation of adenoviral disease [3,16-18], which, in immunocompromised patients, can progress to involve other sites. In one study, 77% of patients with positive adenoviral diarrhea went on to develop viremia [18]. Hepatitis (defined as elevation of hepatocellular enzymes) has been reported to be a potentially severe manifestation of invasive adenoviral disease [19,20]. One of our patients had adenovirus in stool 3 weeks before the onset of adenoviral blood PCR positivity and liver enzyme elevation. Stool positivity might be a predictor for viremia and sometimes disease progression; therefore, especially in the immunocompromised child, preemptive antiviral therapy may be considered. Due to the retrospective nature of our study, PCR positivity was not tested in "real time" and patients did not receive anti-adenoviral treatment. Adenoviral viremia resolved spontaneously in three of four patients despite evidence of invasive disease [Figures 1-3]; however, in one child viremia proceeded rapidly to fulminant disease and death [Figure 4].

Several studies of both adults and children have suggested that T cell depletion is a major risk factor for developing adenoviral infection [6,7]. The present study confirms this finding despite the small number of children tested. The patient who developed disseminated adenoviral infection, which proceeded to multiorgan failure and death, was severely immunocompromised. Another patient who developed invasive adenoviral infection was receiving immunosuppressive medication for GVHD. Several studies have shown that the extent of immune suppression is directly associated with an adverse outcome of adenoviral infection [6-8,21] and recommended withdrawal or reduction of immunosuppressive treatment in patients with proven adenoviral infection [21,22].

Current treatment for adenoviral infection consists mainly of cidovofir and/or ribavirin. Both are of limited efficacy when used to treat disseminated disease [23-25]. The introduction of PCR-based surveillance of adenoviral infection has created an opportunity for early detection and preemptive intervention before the onset of fulminant disease. In this study, PCR in blood became positive at the same time or up to 1–2 weeks before the onset of symptoms; other studies have shown viremia preceding clinical disease by 2–4 weeks. Recently, weekly surveillance of blood for adenovirus using real-time PCR and early intervention with cidofovir resulted in a marked reduction in the disseminated disease rate [18]. Using cidofovir as preemptive treatment proved to be highly effective, with 98% of patients recovering clinically along with the clearing of virus from blood. In another study using a similar surveillance protocol, antiviral treatment and reduction of immune suppression were initiated when blood PCR became positive for adenovirus, resulting in a significantly reduced morbidity and mortality [22].

The extent to which antiviral agents should be used in these

patients remains questionable due to their significant toxicity and to the fact that many children will become asymptomatic with a negative adenoviral PCR without specific treatment, as shown here and by others [5,25]. Still, regardless of treatment strategy, timely diagnosis of adenoviral infection may decrease the number of diagnostic tests performed and may prevent unnecessary treatments (e.g., antibiotics and other antivirals).

This preliminary report suggests that, as reported elsewhere [1-4,17], adenovirus is a significant cause of morbidity in pediatric allogeneic SCT patients, with T cell depletion being a significant risk factor. Molecular diagnosis can be used for routine screening in these patients, facilitating early diagnosis and possibly intervention. Additional studies are required to determine optimal monitoring and treatment strategies and to achieve a better understanding of the clinical course of adenoviral infection in patients undergoing SCT, especially T cell depleted.

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"The easy confidence with which I know another man's religion is folly teaches me to suspect that my own is also"

Mark Twain (1835-1910), American author and humorist

"Commandment Number One for any truly civilized society is this: Let people be different"

David Grayson (1870-1946), U.S. journalist and author