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Original article

Fulminant adenovirus hepatitis after hematopoietic stem cell transplant: Retrospective real-time PCR analysis for adenovirus DNA in two cases

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ABSTRACT

Background: Viral infection is one of the major causes of mortality in patients undergoing hematopoietic stem cell transplant (HSCT). Systemic infection of adenovirus (AdV) has emerged as a not uncommon viral infection with significant morbidity and mortality as with cytomegalovirus and Epstein–Barr virus infection. Routine surveillance for these viruses has become a clinical practice and subsequent pre-emptive therapy improves patients' outcomes; however, the effectiveness of preemptive therapy for AdV has not been fully investigated in patients with a lethal form of AdV infection.

Methods: Sequential AdV loads were retrospectively analyzed in children with fulminant AdV hepatitis after HSCT.

Results: The AdV DNA became detectable (1×10^4 copies/mL) as early as 2 weeks after HSCT. These levels reached $>1 \times 10^8$ copies/mL at the onset of fulminant hepatitis. However, we determined that γ-glutamyltransferase levels were elevated to >100 IU/L at least 2 weeks before the diagnosis of hepatitis.

Conclusions: Our observation raises the possibility that elevated γ-glutamyltransferase could be a sentinel marker for AdV hepatitis, which prompts elaborated monitoring of AdV load and targeted treatment.

1. Introduction

Viral infection has been increasingly recognized as a life-threatening complication in patients undergoing hematopoietic stem cell transplant (HSCT) [1,2]. Among various viruses, cytomegalovirus (CMV) and Epstein–Barr virus (EBV) have been most frequently detected, and the diseases they cause are associated with significant mortality and morbidity. To treat these viral infections as early as possible, routine monitoring of these two viruses by real-time polymerase chain reaction (PCR) is widely applied to patients after allogeneic HSCT [3]. Preemptive therapy for CMV and EBV infection by antiviral agents, rituximab and adoptive T-cell immunotherapy succeed in preventing fatal complications [4,5].

Post-HSCT, adenovirus (AdV) infections present most frequently in organs localized to the respiratory tract, intestine, and urinary system [6]. These infections are readily treated with antiviral agents at the time of definite diagnosis of AdV infection, but the efficacy of antiviral agents including cidofovir is controversial [7]. Several centers provide preemptive therapy by adoptive T-cell transfer for AdV infection [1,8]. In contrast, disseminated AdV infection lacks specific symptoms, which delays diagnosis; therefore, initiation of antiviral therapy. Systemic AdV infection has emerged as a not uncommon viral infection with considerably high mortality rate (5%–83%) [9,10]. AdV infection became the frequent viral cause of mortality at several pediatric transplant centers [1]. Consequently, AdV infection has become emphasized and monitored by real-time

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PCR after allogeneic HSCT; however, preemptive antiviral therapy for low AdV load prevents understanding of the threshold load of AdV, required to cause a life-threatening infection. In the current study, we report on two patients with fulminant AdV hepatitis after HSCT and we assess AdV load and related clinical parameters.

2. Patients and methods

2.1. Patients

All patients undergoing HSCT were weekly monitored for plasma CMV, human herpesvirus 6 (HHV-6), and EBV in our institute. Those patients underwent additional viral workup including AdV who were administered antithymocyte globulin (ATG) and long term steroid, and who had viral-related symptoms such as elevation of liver function tests. Two patients diagnosed with fulminant hepatitis were included in this study. Blood tests including transaminases and γ-glutamyltransferase (GGT) were routinely performed at least three times a week after HSCT. Imaging work up was performed when physical findings and blood tests suggested need for further evaluation. The patients and their parents provided informed consent for routine viral monitoring for CMV, EBV, and HHV-6. Their parents also provided informed consent for retrospective analysis for AdV for research purposes. Sample storage was conducted in accordance with the Declaration of Helsinki. This retrospective study was approved by the ethics committee of the Nagoya University Graduate School of Medicine (approval No. 2014-0054).

2.2. Sample preparation

Before and after HSCT, whole blood samples were routinely collected. DNA was extracted from 200 μL of whole blood using QIAamp DNA Blood Mini Kit (Qiagen, Stanford, CA, USA) and eluted in 50 μL of water. The DNA was used for weekly viral surveillance targeting HHV-6, CMV, and EBV [3], and retrospective assessment targeting AdV.

2.3. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections were deparafinized and underwent antigen retrieval using 10 mM of sodium citrate buffer (pH 7.0) for 30 min at 85 °C. The tissues from the liver were stained with anti-adenovirus type 2 and 5 antibodies [M58 + M73] (ab3648, Abcam, Cambridge, UK) at a 1:50 dilution according to the manufacturer's protocol.

2.4. Electron microscopy

The tissues were fixed in 2.5% glutaraldehyde, postfixed in 2% OsO_4, and embedded in Epon. The sections were stained in uranyl acetate and lead nitrate and were examined with a JEM-1400 transmission electron microscope (JEOL Ltd., Akishima, Japan).

2.5. Real-time PCR

Primers derived from highly conserved AdV hexon 3 and 4 regions were used (5′-GACATGACTTTCGAGGTCGATCCCATGGA-3′ and 5′-CCGGCTGAGAAGGGTGTGCGCAGGTA-3′) [11]. These primers are confirmed to detect AdV types 1, 2, 3, 4, 5, 6, 7, 8, and 11. Real-time PCR was performed as described previously [12], with some modifications. In brief, PCR was performed in a total volume of 25 μL, including 2 μL of extracted DNA, 1× SYBR Premix Ex Taq (Takara Bio, Otsu, Japan) and 160 nmol/L of each primer. Amplifications were conducted using an Mx3000P Real-Time PCR System (Agilent Technologies, Santa Clara, CA) with 50 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, and elongation at 72 °C for 1.5 min. AdV serotype 2 PCR product cloned into the pCR2.1 plasmid was used for quantification standard.

3. Results

3.1. Case presentation: patient 1

A 12-year-old female with acute myeloid leukemia (AML FAB M1) received bone marrow transplantation (BMT) from an HLA-matched sibling donor during her second complete remission (CR). Two years later, she experienced relapse and underwent haploidentical HSCT from her mother during her third CR. She received fludarabine (30 mg/m^2 once daily i.v. for 4 days), melphalan (70 mg/m^2 once daily i.v. for 2 days), and total body irradiation (3 Gy) as a conditioning regimen combined with rabbit ATG (2.5 mg/kg once daily continuous i.v. for 4 days). For GVHD prophylaxis, tacrolimus and short-term methotrexate were administered. In addition, she received prophylactic acyclovir, fluconazole, and weekly immunoglobulin.

On day +26, she had fever with mildly elevated liver enzymes without evidence of acute GVHD. She was treated with methylprednisolone (2 mg/kg/day) under a diagnosis of engraftment syndrome. On day +28, neutrophil engraftment was achieved and she became afebrile. The transaminase levels gradually normalized and methylprednisolone was tapered. She had fever and grade 2 diarrhea on day +78, and did not respond to antibiotics or antifungal agents. She was considered to have acute GVHD (grade III) and a response was obtained using methylprednisolone (started at 1 mg/kg/day for 7 days, tapered and maintained at 0.5 mg/kg/day). She had fever again on day +91 with an elevation of EBV burden (21,915 copies/mL) and preemptive rituximab was administered (375 mg/m^2), which resulted in disappearance of EBV load a week after treatment commenced, although her fever remained as high as 39.0 °C.

On day +103, total bilirubin, aspartate transaminase, and alanine transaminase suddenly elevated to 6.7 mg/dL (normal range: 0.3–1.2 mg/dL), 584 IU/L (normal range: 9–38 IU/L), and 447 IU/L (normal range: 4–36 IU/L), respectively. The absolute lymphocyte count was 0.1 × 10^9/L. No response was obtained using antimicrobial or corticosteroid therapy. On day +105, she had unmanageable epistaxis and was admitted to the intensive care unit. An enhanced abdominal CT scan revealed multiple patchy, non-enhanced lesions inside hepatic parenchyma (Fig. 1a). Bacterial and fungal cultures from blood, urine, sputum, and stool were negative. In addition, serological analysis for hepatitis A, B, and C viruses and real-time PCR for CMV, EBV, and HHV-6 were negative. On day +108, she was diagnosed with fulminant hepatitis according to the published criteria [13], and received 5 mg/kg cidofovir and donor lymphocyte infusion (DLI) for the presumptive diagnosis of AdV infection. Despite intensive supportive care, she died of hepatic failure on day +113. Viral cultures were positive for AdV serotype 2 in plasma, urine, and throat secretions obtained before the administration of cidofovir.

3.2. Case presentation: patient 2

A 16-year-old female was diagnosed with severe aplastic anemia and received immunosuppressive therapy (IST) with ATG and cyclosporine A. She did not respond to IST, and was referred to our hospital for BMT from an HLA-matched unrelated donor 1 year after IST. She received fludarabine (25 mg/m^2 once daily i.v. for 5 days), melphalan (70 mg/m^2 once daily i.v. for 2 days), and total body irradiation (7.5 Gy, 2 fractions) as a conditioning regimen