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ORIGINAL ARTICLE

Disseminated Adenovirus Disease in Immunocompromised Patient Successfully Treated with Oral Ribavirin: A Case Report

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Abstract In patients with immunological disorders, adenovirus infections are associated with significant rates of morbidity and mortality. Only few hematological units use molecular virological methods, such as polymerase chain reaction, for surveillance of adenovirus infection, and treatment strategies have never been evaluated in multicenter clinical trials. This report describes the detection and treatment of human adenovirus (HAdVs) disseminated disease in the case of a 46-year-old immunocompromised female having myelodysplastic syndrome with refractory cytopenia with multilineage dysplasia: International Prognostic Scoring System 1. Serum and urine samples were tested for the presence of adenoviral DNA using the quantitative real-time polymerase chain reaction (PCR) assay. For additional confirmation, sequencing of PCR products was also performed. With real-time PCR, we detected HAdV DNA in both serum and urine samples. The viral level constantly decreased with applied oral ribavirin therapy. As the result of sequencing, HAdVs type 11 was determined. Surveillance of adenovirus by real-time PCR is useful in detecting and monitoring disseminated HAdV infection; it is a potential standard diagnostic approach that could assist clinicians to decide whether antiviral therapy ought to be administered.

Keywords Adenovirus infection · Immunosuppression · Myelodysplastic syndrome · Real-time PCR

Abbreviations

HAdV	Human adenovirus
MDS	Myelodysplastic syndrome
CMV	Cytomegalovirus
EBV	Epstein-Barr virus
HSV-1/2	Herpes simplex virus type 1/2
VZV	Varicella-zoster virus
HHV-6	Human herpesvirus type 6

Introduction

Human adenoviruses (HAdV) are a group of non-enveloped, double-stranded DNA viruses, which are endemic worldwide. Adenoviral infections in human may result in variety of clinical symptoms, including respiratory disease, epidemic keratoconjunctivitis and gastroenteritis (Ruuskanen et al. 2002). Currently, 55 serotypes of HAdV are known, which are grouped into seven species (A–G) (Walsh et al. 2010). Especially, serotypes from species A, B and C are commonly recognized as pathogens causing severe morbidity and mortality in immunosuppressed patients. In these cases, clinical signs of an adenoviral infection are rather non-specific and variable, including pneumonia, gastroenteritis, hemorrhagic cystitis and neurological infections (Kojaoghlanian et al. 2003; Ruuskanen et al. 2002) Table 1.

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Table 1 Changes in clinical parameters between days 0 and 80 after patient's admission to Department of Hematology, Oncology and Internal Diseases

Parameter	Day 0	Day +14	Day +21	Day +28	Day +35	Day +49	Day +56	Day +70	Day +80
PLT count ($\times 10^3$ μl^{-1})	23.0	14.0	9.0	13.0	8.0	28.0	14.0	18.0	9.0
WBC count ($\times 10^3$ μl^{-1})	1.4	1.8	1.3	0.9	1.1	1.0	1.0	1.8	1.4
HGB (g/dl)	11.1	10.0	10.5	12.2	11.0	8.9	7.4	10.7	7.4
CRP (mg/l)	100	169.4	130	9.3	7.5	9.7	26.6	11.4	N/A
Creatinine, serum level (mg/dl)	0.76	0.42	0.46	0.49	0.42	0.44	N/A	0.64	N/A
Proteins, urine level (mg/dl)	65	>2	36	43	40	32	N/A	28	N/A

N/A not available, *PLT* platelets, *WBC* white blood cell, *HGB* hemoglobin

Polymerase chain reaction (PCR), as well as its variant, real-time PCR (qPCR), has become a valuable tool for the detection of adenoviral DNA due to its speed, specificity, and sensitivity which allow for early diagnosis (Bil-Lula et al. 2010a; Rola et al. 2007). Quantitative or semi-quantitative PCR assays allow also monitoring of the kinetics of the viral infection or effectiveness of antiviral treatment (Claas et al. 2005). Quantification of HAdV DNA load has been suggested especially in patients who are at the greatest risk of dying from disseminated adenoviral infection (Bil-Lula et al. 2010b; Claas et al. 2005; Lion et al. 2003), but treatment strategies supported with quantitative results of virus load monitoring with real-time PCR have not been validated by clinical trials so far.

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoietic stem cells that are characterized by ineffective hematopoiesis, multilineage dysplasia, peripheral cytopenia, and susceptibility to leukemic transformation (Nimer 2008). Owing to anemia and dysfunction of the white blood cells because of the immunodeficiency, the patients require frequent transfusions. The most frequent causes of death in these patients are septic complications or bleeding secondary to the thrombocytopenia. Allogeneic transplantation of blood stem cells is the only curative treatment for MDS. Because of the older age of patients with MDS and high mortality and morbidity rates associated with graft-versus-host disease, transplantation has generally been reserved for patients with high-risk MDS or MDS transforming to leukemia (Nimer 2008). The International Prognostic Scoring System relies on the primacy of the number of cytopenias, cytogenetic profile, and the percentage blasts in the bone marrow in order to group patients with MDS into one of the four prognostic categories: low risk, intermediate 1 risk, intermediate 2 risk, and high risk.

In our opinion, this work is one of the few virology-confirmed cases of adenoviral infection in an immunocompromised patient, and the first successful treatment of HAdV disease in Poland.

Case Report

A 46-year-old woman with a preliminary diagnosis of macrocytic anemia (2000), subsequently verified in 2005 by a histopathological examination (trepanobiopsy) as MDS with refractory cytopenia with multilineage dysplasia (MDS-RCMD), remains under the constant care in the Department of Hematology since 2005. The patient was treated symptomatically (transfusions of red blood cell and platelet concentrates), due lack of consent for treatment with bone marrow transplantation. The presence of lymphocytotoxic antibodies (detected in 2008) was an additional problem in the management of the patient.

The patient was hospitalized since 09.03.2010 with severe hematuria, intense lower abdominal pain, and a continuous feeling of urgency to urinate, and urine dripping. On physical examination, the urinary bladder was extended up to the umbilicus. Transfusion support was continued, and at the same time we tried to indentify the cause of hematuria. In sonography no major difference in comparison to the previous examination was found, apart from a low degree of urine stasis in the right kidney and non-homogeneous hypoechogenic content in the lumen of the urinary bladder, most probably representing blood clots. Urine culture revealed mixed flora of Gram(+) cocci assessed as a contamination. Repeated sonography results revealed poorly filled urinary bladder with hyperechogeneous regions (air bubbles and blood clots). In computed tomography (CT) scan of the abdominal cavity, the thickness of the wall of the whole urinary bladder was increased in the early vascular phase of contrast-enhanced image of mucosa; no tumorous mass was detected. Due to mild hospital-acquired transient diarrhea, low-grade fever and high levels of C-reactive protein (CRP; 168 mg/dl max) wide-range microbiological diagnostics was performed. Additionally acquired CT scan of the lungs showed a lesion in the right lung to be differentiated with a fungal lesion; oral itraconazole in typical doses was started. Blood samples were also checked for presence of genetic material of cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes

simplex virus type 1/2 (HSV-1/2), varicella-zoster virus (VZV), and human herpesvirus type 6 (HHV-6) and turned out negative. Bone marrow assessment excluded blastic transformation as the cause of cytopenias and fever.

After 4 weeks since the onset of the hematuria associated with severe lower abdominal pains, urgency to urinate and continuously rising CRP levels, patient presented first episode of generalized convulsions, with eyeballs shifted to the left, lasting approximately 20 s. A CT of the central nervous system showed no signs of bleeding; electroencephalography was normal. After a second episode of generalized convulsions, the consulting neurologist ordered prophylactic treatment with carbamazepine. Magnetic resonance imaging scans showed an abnormality: a couple of lesions in the white matter of the both hemispheres, which might correspond to viral neuroinfection or ischemic changes. Due to general contraindications to cerebrospinal fluid collection (thrombocytopenia $<50\times10^3/\mu l$), assessment of possible viral infections was performed only in serum (CMV, VZV, HSV-1/2, EBV, HHV-6, and HAdV): all were negative except for adenoviruses. As the positive results of urine testing for adenoviral DNA supported the diagnosis of viral infection, the treatment with oral ribavirin was initiated. On day +35, nitrofurantoin-sensitive *Escherichia coli* was detected in a single urine sample, and normal adult dose of nitrofurantoin was administered (100 mg three times daily for 10 days). After 2 weeks of treatment, the urinary catheter was removed; the patient presented no hematuria or dysuric symptoms. The patient was discharged on day +40, receiving prophylaxis with carbamazepine, acyclovir, co-trimoxazole and ribavirin. In control sonography of the urinary bladder made on day +70, no abnormalities were found. Therapy with ribavirin was disrupted on day +80 following negative HAdV qPCR results both in serum and urine. Currently, the patient is under care of the outpatient clinic in satisfying general status, still requiring blood transfusion support, with no recurrence of adenoviral infection so far.

Materials and Methods

Specimens for virological examinations were taken for 6 weeks and whole DNA was isolated from serum and urine samples using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany). The following viruses were tested: HSV-1/2, VZV, EBV, CMV, HHV-6 and HAdVs. Examinations were performed using real-time PCR technique in the LightCycler 2.0 instrument. All tests were produced by Roche Diagnostics, except *"in-house"* kits for HHV-6 and HAdVs detection (Dzieciątkowski et al. 2008; Rola et al. 2007).

End-point PCR (Echavarria et al. 1998), producing 308 bp-long amplicon (hexon gene) was performed with DNA isolated from adenovirus-positive urine and serum samples. PCR products were treated with shrimp alkaline phosphatase and *E. coli* exonuclease I (MBI Fermentas, Lithuania) to dephosphorylate dNTPs and to digest PCR primers. Next, labeling with v 3.1. Chemistry (Applied Biosystems) was performed using both 5′ and 3′ PCR primers, with subsequent purification of the products with BigDye Xterminator set (Applied Biosystems, USA). Purified sequencing products were electrophoresed in ABI 3130 capillary analyzer (Applied Biosystems, USA). Obtained sequences were compared with the NCBI database, and homologous sequences with the highest matches were considered as identification results.

Retrospectively, IgG and IgM antibody levels against HAdVs were measured in first and last serum specimen, using a commercial qualitative enzyme immunoassay (NovaTec, Germany).

Results

Microbiological Findings and Patient's Management

All results of virological findings (CMV, VZV, HSV-1/2, EBV, and HHV-6), were negative in all examined samples, except for HAdVs. The adenoviral DNA was detected in six of eight serum samples and in seven of eight urine samples in the considered period. Level of HAdV DNAemia was constantly decreasing, ranging from 820 copies/ml in first examined serum sample to 160 copies/ml in the last positive sample and from 46,500 to 3,800 copies/ml in urine samples, respectively (Fig. 1). Based on clinical signs and the positive results of HAdV DNA detection in

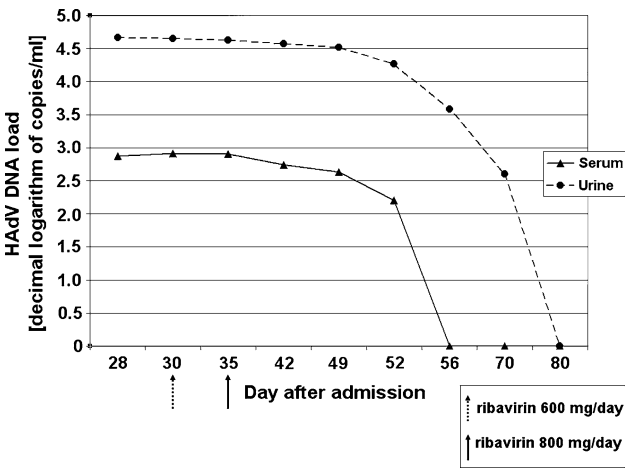

Fig. 1 Changes in HAdV DNA load in serum and urine samples

patient's sera and urine samples, a disseminated adenovirus infection was diagnosed.

In further investigations as the result of sequencing part of adenoviral gene encoding hexon protein a HAdVs type 11 (species B) was determined, both in urine and serum samples.

Specific IgM antibodies were negative in first serum sample and equivocal in sample taken day +56. Anti-adenovirus IgG were positive in both samples described above.

Treatment with use of oral form of ribavirin (600 mg/day, increased to 800 mg/day after 5 days) was introduced in fourth week after the presentation of first clinical symptoms. Patient status constantly improved and decreasing level of HAdV DNA copies in routine testing both in urine and sera samples was observed.

Discussion

The role of adenoviruses in the morbidity and mortality of immunocompromised patients is being increasingly recognized (Kojaoghlanian et al. 2003; Lion et al. 2003). Possible mechanisms of infection include primary respiratory route, faecal-oral transmission or reactivation of a virus causing persistent asymptomatic infection. The broad spectrum of clinical presentations in HAdV infections warrants necessity for virological testing of samples from individuals with compatible clinical syndromes (Ruuskanen et al. 2002). Hemorrhagic cystis associated with HAdV 11 excretion in urine has been described in immunosuppressed individuals with significant risk factors as female gender and seropositivity for the antibodies to adenoviruses. The disease lasted for 4–6 weeks and was associated with hematuria, urinary frequencies, burning urination and fever (Akiyama et al. 2001; Watcharananan et al. 2011).

Currently, there is no established antiviral therapy for adenovirus infections (Lenaerts and Naesens 2006; Lenaerts et al. 2008). Successful treatment of HAdV infections with intravenous form of ribavirin has been noted (Homma et al. 2008), however, treatment failures have also been reported (Chakrabarti et al. 1999). Adenovirus detected by real-time PCR in the urine and serum of described patient was identified by sequencing as species B (serotype 11). Available data supports thesis about lack of ribavirin efficacy against adenoviruses belonging to B species in in vitro experiments (Morfin et al. 2005, 2009) with exception for HAdV B (serotype 34), which demonstrated sensitivity to ribavirin in experiments driven by a single research center (Stock et al. 2006).

Cidofovir, a broad-spectrum antiviral agent proven for the treatment of CMV infections, has been also found to be effective against all adenoviruses in vitro (Morfin et al. 2009) and treatment with cidofovir was associated with a favorable outcome in some reports of serious adenovirus disease in immunocompromised patients (Lindemans et al. 2010; Neofytos et al. 2007). Also successful use of cidofovir and intravenous immunoglobulin in renal transplant patients with disseminated HAdV infection has been described (Saquib et al. 2010). Unfortunately, the toxicity of this drug is considerable (Lenaerts and Naesens 2006).

Treatment of adenoviral infections with use of both antiviral drugs depends mostly on HAdV load in clinical specimens and decreases along with time past during the infection (Lenaerts et al. 2008; Lion et al. 2003). In these situations, qPCR assay became very useful tool for the rapid, specific, and sensitive detection of HAdVs in different kind of samples (Bil-Lula et al. 2010b). In addition to the promptness of detection, determination of the viral load is valuable because it may have predictive value for disseminated adenoviral disease (Claas et al. 2005; Lion et al. 2003). In viral hemorrhagic cystitis with concomitant signs of disseminated infection, real-time PCR technique may also benefit in discrimination between infections caused by adenoviruses, polyomavirus BK and herpes simplex viruses, with regard to different treatment strategies used in infections with these viruses (Bil-Lula et al. 2010a). Infections caused by adenoviruses should be also included in the differential diagnosis of a wide variety of clinical syndromes, including pneumonia, enteritis, hemorrhagic cystitis, conjunctivitis, and disseminated disease. The highest mortality rates were observed among patients with pneumonia and disseminated disease (Lion et al. 2003; Omar et al. 2010). HAdV disease still poses a challenge to clinicians, who are vested only with therapeutic options of limited efficacy for this infection. All the more, the possibility of monitoring an infection course by quantitative detection of adenoviral DNA is supportive in the decision for both introduction and withdrawal of the antiviral agent. Taken together, the features of this assay may contribute to substantial improvement of the surveillance and clinical outcomes of patients at risk for adenoviral infections.

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