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LETTER TO THE EDITOR

Human adenovirus infection in patients subjected to allogeneic hematopoietic stem cell transplantation – a three-year single center study

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Human adenoviruses are increasingly recognized as pathogens causing life-threatening infections in immunosuppressed patients, especially after allogeneic hematopoietic stem cell transplantation (HSCT) (*1*). Species A, B, and C are commonly recognized as pathogens causing severe morbidity and mortality in immunosuppressed patients (*2*). Clinical signs of an adenoviral infection are rather nonspecific and variable, including: tonsillopharyngitis, pneumonia, gastroenteritis, hepatitis and hemorrhagic cystitis (*1–2*).

PCR, or its variant real-time PCR, has become a valuable tool for the detection of adenoviral DNA due to its speed, specificity, and sensitivity, which allow for early diagnosis (*3*). Quantification of human adenoviruses (HAdV) has been suggested especially in patients who are at greatest risk of dying from disseminated adenovirus infection (*4*), and for effectiveness of antiviral treatment (*4-5*), but treatment strategies based on the quantification of virus load with real-time PCR have not been validated by clinical trials so far (*5*).

In the current study, using quantitative real-time PCR, we estimated the frequency of adenoviral infections in 57 consecutive hematological patients who had undergone allogeneic HSCT.

IgG and IgM antibodies specific for human adenoviruses were measured in a panel of patients' serum specimens, using NovaLisa®, a commercial qualitative enzyme immunoassay test (NovaTec Immunodiagnostica). Serological examination was performed once, with the serum sample collected prior to HSCT. Real-time PCR was used for detection of adenoviral DNA in 451 sera samples, which were being collected once a week, until 100th day after transplantation. Real-time PCR tests were run on LightCycler 2.0 instrument (Roche Diagnostics), using a modified *"in-house"* quantitative method (*3*), which allows detection of all known groups of human adenoviruses.

End-point PCR, amplifying 197 bp-long product (a fragment of the hexon gene) (*6*), was performed with nucleic acids isolated from serum samples, in which adenoviral DNA was detected by real-time PCR. Purified end-point PCR products were used for sequencing in ABI 3130 capillary analyzer (Applied Biosystems). Obtained sequences were compared to the NCBI database, and homologous sequences with the highest score were considered as the identification results of HAdV species.

HAdV-positive patients were predominantly middle-aged (median 36 years), and males outnumbered females. Adenoviral DNA was detected in 47 sera samples (10.4%) and viremia was observed between day 19 and 70 after transplantation. Sequencing of the part of adenoviral hexon protein gene identified detected adenoviruses as belonging to group B (in 82% of the patients) and C (in 18% of the patients).

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Abbreviations: GvHD = graft-versus-host disease; HAdV = human adenoviruses; HHV-5 = human herpesvirus 5; HSCT = hematopoietic stem cell transplantation

Table 1. Demographic and virological data of patients from the study group

Total number of patients (males/females)	57 (27/30)
Number (%) of patients positive for anti-HAdV IgM	1 (2%)
Number (%) of patients positive for anti-HAdV IgG	54 (95%)
Number (%) of HAdV DNA positive patients	11 (19.3%)
Detected groups of HAdV (%)	B (82%) / C (18%)
Average HAdV load (copies/ml)	4.32×10^3
Range of HAdV DNA load (copies/ml)	1.12×10^2 –2.86×10^4

Seven HAdV-positive transplant recipients developed pneumonia 14 to 87 days post HSCT (median 49 days), but in five of them parallel human herpesvirus 5 (HHV-5) infection was detected. Testing for HHV-5 was conducted with use of CMV Quant Kit® (Roche Diagnostics), according to EBMT guidelines (*7*), as part of routine post-transplant monitoring of the patients. All patients from described group had abnormal radiological findings, but there was no consistent abnormality on chest X-ray.

The role of adenoviruses in the morbidity and mortality of immunocompromised individuals is being increasingly recognized (*1*–*5*). Possible means of infection include primary respiratory route, fecal-oral transmission or reactivation of a virus persistent in the body.

Using real-time PCR, we found that 19.3% patients in our study group had adenoviral DNA in serum samples. This is slightly lower frequency than in other studies describing HAdV incidence as 19.7% (*8*), or 27.0% (*5*). The majority of HAdV-positive transplant recipients had fever of unknown etiology within the first 10 weeks after HSCT. The presented results indicate that adenoviruses are seldom disseminated to peripheral blood of patients with infections of lower respiratory tract.

Importantly, HAdV infection may cause diagnostic confusion, particularly mimicking some of the symptoms of gastrointestinal graft-versus-host disease. Diarrhea is the most common presentation of those symptoms. The relationship between adenoviruses and graft-versus-host disease (GvHD) still remains unclear. The two may coexist, but the viral infection may also be a trigger for GvHD. On the other hand, the immunosuppression associated with GvHD and its therapy may increase the probability of symptomatic adenoviral infection. Shields *et al.* found such an association (*9*), but Baldwin *et al.* (*10*) found the incidence of acute GvHD at a similar level in groups of patients (transplant recipients from unrelated donor) both with and without adenoviral infections.

Currently, there is no established antiviral therapy for adenovirus infections (*11*). Successful treatment of isolated HAdV pneumonia with ribavirin has been noted (*12*), however, treatment failures have also been reported (*13*). Cidofovir, a broad-spectrum antiviral agent proven for the treatment of HHV-5 disease, has been also found to be effective against all adenoviruses *in vitro* (*14*), and treatment with cidofovir was associated with a favorable outcome in some reports of serious adenovirus disease in immunocompromised patients (*11*). A small number of publications have demonstrated a link between the recovery of endogenous adenovirus-specific T-cells and protection against infection and disease *in vivo*. Consequently, protocols for the infusion of *in vitro* expanded adenovirus-specific T-cells as prophylaxis and/or treatment of infection and disease are still being developed (*12*).

In summary, the presented real-time PCR assay is a tool for rapid, specific, and sensitive detection of HAdVs in clinical samples. In addition to the speed of detection, determination of the viral load is valuable, because it may have predictive value for disseminated adenoviral disease (*5*). Application of this method in clinical virus screening could therefore contribute to the improvement of the outcome of HAdV infections in immunocompromised patients.

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