

# Incidence of Adenoviral DNAemia in Polish Adults Undergoing Allogeneic Haematopoietic Stem Cell Transplantation

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**Abstract** Human adenoviruses (HAdV) are important viral pathogens recognized increasingly in immunocompromised hosts, especially in allogeneic haematopoietic stem cell transplant recipients (alloHSCT). The clinical spectrum of HAdV disease ranges from asymptomatic viraemia and mild self-limiting disease to lower respiratory tract infection, multi-organ involvement and even death. Early detection and quantification of HAdV in peripheral blood using real-time PCR (qPCR) assay has been suggested as a useful monitoring tool, but is seldom used for regular surveillance of HAdV in haematology centers. A group of 112 alloHSCT recipients from two hospitals in Warsaw (Poland) was examined in the early post-transplant period using a quantitative qPCR assay. A total of 1,245 serum samples were evaluated for presence of HAdV DNA in patients where 66 (59 %) patients received grafts from unrelated donors whereas the other 46 (41 %) from sibling donors. HAdV sequences were detected in 64 (57 %) of the

112 patients. In 22 of all patients (20 %) HAdV DNA was detected only in a single positive sample, while 42 (37 %) had positive results in two or more subsequent sera. In total, DNAemia was present in 202 sera samples (16 %) with median time to observation of 47 days. Graft-versus-host disease (GvHD) was observed in 18 (28 %) adenovirus-infected transplant recipients and a significant correlation between HAdV infections and GvHD clinical presentation was found ( $p = 0.018$ ). There is a high prevalence of HAdV infections in HSCT recipients in Poland during early post-transplant period. In consequence, we could only speculate if HAdV DNAemia could be also related to GvHD symptoms, enforcing the important pathogenic role of these viral infections in clinical complications post-alloHSCT.

**Keywords** HAdV ·  
Haematopoietic stem cell transplantation ·  
Post-transplant infectious complications · Real-time PCR

## Abbreviations

alloHSCT	Allogeneic haematopoietic stem cell transplants
alloPBSCT	Allogeneic peripheral blood stem cell transplantation
FUO	Fever of unknown origin
GvHD	Graft-versus-host disease
HAdV	Human adenoviruses
qPCR	Real-time PCR

## Introduction

Human adenoviruses (HAdV) are non-enveloped, double-stranded DNA viruses which cause ubiquitous infections

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worldwide (Fischer 2008). Infection in children and immunocompetent adults is usually benign, manifesting most commonly as an upper respiratory tract infection or gastroenteritis. Severe HAdV infections, leading to multiple organ manifestations and fatal outcome have been reported in patients with congenital immunodeficiency disorders, with AIDS and in recipients of solid organ or allogeneic haematopoietic stem cell transplants (allo-HSCT) (Kojaoghlanian et al. 2003). According to different studies, the estimated rate of HAdV infection after HSCT ranges 3–47 % with high mortality ranging 6–86 % (Bil-Lula et al. 2010; Sive et al. 2012; Taniguchi et al. 2012). In transplant recipients, HAdV infection may have occurred de novo by newly acquired infection from donor, other exogenous sources, or from reactivation of persistent (latent) infection. Suggested risk factors for HAdV infection include young age of recipients (Baldwin et al. 2000), grade II–IV graft-versus-host disease (GvHD), unrelated or mismatched transplantations (Suparno et al. 2004), profound T cell depletion of the graft, anti-thymocyte globulin treatment (Matthes-Martin et al. 2012), bone marrow as the stem cell source, myelodysplastic syndrome as indication for transplantation (Öhrmalm et al. 2011) and detection of rising HAdV viral load in blood (Lion et al. 2003) or in stool samples (Lion et al. 2010).

Early detection of HAdV infection and identification of patients at high risk for HAdV disease are relevant. HAdV infections are not easily diagnosed and the development of a severe infection is hard to diagnose by standard culture techniques (Lankester 2007). Following HSCT, immunological responses to HAdV are poor; hence serological diagnosis is not reliable. Molecular identification of HAdV infection with PCR facilitates accurate and rapid diagnosis and can be used also for surveillance (Bil-Lula et al. 2010; Öhrmalm et al. 2011). Weekly monitoring of different fluids of transplant recipients for active HAdV infection by real-time PCR (qPCR) could potentially be applied in HSCT recipients (Bil-Lula et al. 2010; Matthes-Martin et al. 2012). Treatment of HAdV infection using ribavirin and/or cidofovir has not been proven in controlled trials yet, and their administration is limited by toxicity in adults (Lenaerts and Naesens 2006; Matthes-Martin et al. 2012).

At present, surveillance of HAdV infection in patients after alloHSCT is not implemented routinely (Bil-Lula et al. 2010), although proper qPCR assays are widely available. The aim of the present study was to evaluate the incidence and frequency of HAdV DNAemia in serum samples of Polish adult alloHSCT recipients and to correlate clinical symptoms with DNA positivity. Better understanding of the incidence and outcomes of HAdV infections after alloHSCT may be the basis for developing strategies for more effective treatment.

**Table 1** Characteristics of patients ( $N = 112$ )

Median age in years (range)	41 (18–65)
Gender	
Male	59
Female	53
Primary disease	
Acute myeloid leukemia	53
Acute lymphoblastic leukemia	19
Myelodysplastic syndrome	9
Aplastic anemia	6
Chronic myeloid leukemia	5
Non-Hodgkin lymphoma	5
Hodgkin's lymphoma	3
Myeloproliferative neoplasms	3
Chronic lymphocytic leukemia	2
Chronic myelomonocytic leukemia	2
T cell prolymphocytic leukemia	2
Multiple myeloma	2
POEMS syndrome	1
Stem cell donor	
Unrelated	66
Related	46
Conditioning regimen	
Myeloablative	76
Using anti-thymocyte globulin	34
Without using anti-thymocyte globulin	42
Non-myeloablative	36
Using anti-thymocyte globulin	17
Without using anti-thymocyte globulin	19
Anti-GvHD prophylaxis	
Cyclosporine + methotrexate	78
Cyclosporine + mycophenolate mofetil	34

## Materials and Methods

This retrospective study involved 112 adult patients, who underwent consecutive allogeneic peripheral blood stem cell transplantation (alloPBSCT) in the Department of Haematology, Oncology and Internal Medicine, Medical University of Warsaw and in the Department of Haematopoietic Stem Cell Transplantation, Institute of Haematology and Transfusion Medicine between 2009 and 2012. Additional criteria for statistical analysis included appearance of at least one from syndromes listed below in early post-transplant period: development or progression of GvHD, lower respiratory tract infection or neutropenic fever. Monitoring of clinical status of all the patients and viral load in serum samples covered the period of 100 days after HSCT and mortality rates were analyzed for 1 year after HSCT. The characteristics of patients are shown in Table 1.

**Table 2** Sequences of primers and probe used in this work

Name	Sequence (5'–3')
AdenoF	GGA CGC CTC GGA GTA CCT GAG
AdenoR	ACA GTG GGG TTT CTG AAC TTG TT
Adeno_P	JOE–CTG GTG CAG TTC GCC CGT GCC–BHQ-2

Presence of antibodies specific for HAdV, both in IgM and IgG classes, was measured in a panel of serum specimens coming from all of the patients, using a commercial qualitative NovaLisa™ ELISA tests (NovaTec Immunodiagnostica, Germany), according to the manufacturer's instructions. Examination was performed once, with the serum sample collected prior to HSCT.

The median number of serum specimens per patient was 11 (range 1–18). A total number of 1,245 samples were examined using qPCR assay. Total DNA was extracted from 200 µl of serum, using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany), in accordance to the manufacturer's instructions.

Real-time PCR tests were run on LightCycler 480 instrument (Roche Diagnostics, Germany), using a modified “in-house” quantitative method, described below (Rola et al. 2007). Highly conservative region encoding hexon protein gene has been chosen (GenBank, AC 000008), and set of primers was developed, as well as the probe, labeled with fluorophore reporter JOE on 5' end and with BHQ-2 quencher on its 3' end (Oligo, Poland) (Table 2). Investigations were performed using reaction mixture TaqMan Master Kit® (Roche Diagnostics, Germany), which contained, besides chemicals supplied by kit producer, 5 µl of isolated DNA, 0.75 µM of both primers and 1.50 µM probe in total volume of 20 µl. Amplification was performed with activation of thermostable hot-start DNA polymerase for 10 min at 95 °C, followed by 40 cycles comprising denaturation (15 s at 95 °C), primers annealing (10 s at 60 °C) and strand elongation (10 s at 72 °C). Each amplification reaction embraced, except tested samples, also positive HAdV controls in range 100–100,000 copies/ml and negative control of DNA extraction and amplification process. Fluorescence levels were measured at 560 nm wavelength and a threshold cycle ( $C_t$ ) value for each sample was calculated.  $C_t$  values of HAdV calibrators were the basis for standard curves and the copy numbers were calculated automatically by a software package for data analysis.  $C_t$  values of tested clinical samples  $\leq 35.00$  were considered as positive. As specific international threshold values of HAdV viraemia have not been defined, limit of detection of used qPCR assay, established on level 100 copies/ml, was adopted for this study as a “cut-off” value.

**Table 3** Virological characteristics of the HAdV-positive patients ( $N = 64$ )

	Patients with HAdV DNAemia
Median of HAdV detection (days post HSCT)	47
Range of HAdV detection (days post HSCT)	1–100
Median of HAdV-positive serum samples	3
Range of HAdV-positive serum samples	1–11
Median of HAdV DNA quantity (copies/ml)	618
Range of HAdV DNA quantity (copies/ml)	100–28,600

All statistical analyses were performed using Statistica 10 software package (StatSoft, Inc.).  $\chi^2$  tests were used for univariate comparisons to examine categorical variables, including age, primary underlying disease and donor type. The Mann–Whitney  $U$  test was applied to examine the medians between groups. Survival curves were generated by the Kaplan–Meier method.  $P$  values  $< 0.05$  were considered statistically significant.

## Results

Specific IgM class anti-HAdV antibodies have not been detected in any patient before alloHSCT. One hundred and seven patients (96 %) had IgG antibodies against HAdV, while five were negative (4 %). None of IgG-negative patients developed detectable HAdV DNAemia during early post-transplant period.

HAdV DNA was detected in 64 of 112 patients (57 %). In 22 of them (20 %) HAdV DNA was detected only in a single positive sample, while 42 (37 %) had positive results in two or more subsequent tests. In total HAdV sequences were detected in 202 sera samples (16 %) and the median time to day of first HAdV DNA detection was 47 days (range 1–100 days). The median copy number was 618; range 100–28,600 copies/ml (Table 3). The infections did not show any seasonal variation. Additional statistics showed that donor type ( $p = 0.263$ ) and primary underlying disease ( $p = 0.103$ ) had no effect on the HAdV infection in studied group. Statistical significance was shown for association between HAdV DNAemia and development of GvHD ( $p = 0.018$ ) and age of patients ( $p = 0.022$ ; Table 4). Comparison of variables between HAdV-positive and HAdV-negative patients is shown in Table 5.

**Table 4** Frequency of adenoviral infections in different age groups

Age in years (range)	Number of patients	Proportion of HAdV-infected patients (%)
18–25	19	58
26–35	23	66
36–45	20	65
46–55	27	55
56–65	23	48

Analysis of clinical course of patients with HAdV DNAemia showed that among 64 patients who had HAdV infections, 18 (28 %) developed GvHD (grade I–II) compared to the patients without HAdV DNAemia where only five (8 %) developed GvHD (grade I–II) ( $p = 0.022$ ). GvHD symptoms were observed after detectable HAdV DNAemia with median 6 days (range 3–10 days) in 15 out of 18 patients with GvHD and HAdV DNAemia. Patients with GvHD showed significantly higher median viral load (1,161 copies/ml), compared to patients without GvHD (205 copies/ml);  $p = 0.004$ . Symptomatic infections manifested usually as upper and/or lower respiratory tract infections and rarely gastroenteritis. Pyrexia was present in 46 (72 %) patients with HAdV infection and often was noted as fever of unknown origin (FUO). The most common respiratory tract symptom was cough (11 %). Two patients had bronchitis (3 %) and three patients (5 %) developed pneumonia. Diarrhea was reported in three

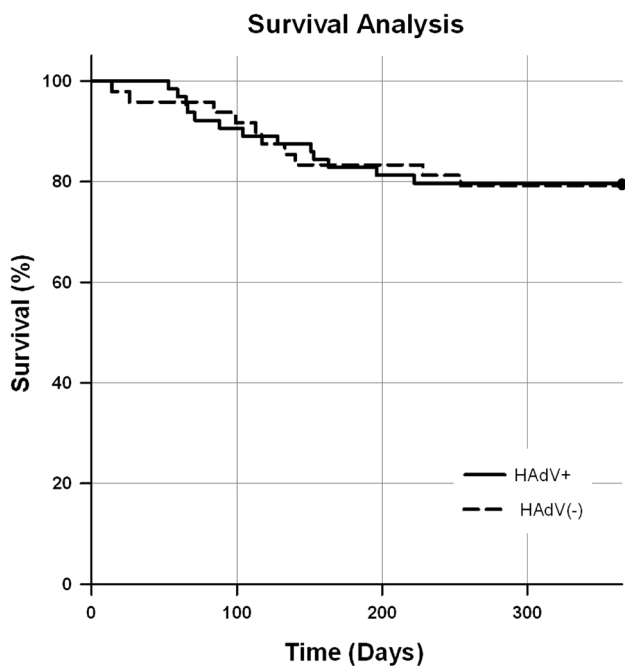
patients (5 %) and symptoms of hemorrhagic cystitis were observed in 11 patients (17 %). Clinical symptoms described above could be associated with HAdV infection, as the most results of the other routinely provided microbiological investigations were considered in this time as negative. Fifteen (23 %) patients had HAdV DNAemia without any clinical manifestations. No correlation was observed between viraemia level and the clinical symptoms during HAdV infection ( $p = 0.998$ ), between the presence of HAdV DNA and conditioning regimen (myeloablative vs. non-myeloablative;  $p = 0.294$ ), or between HAdV infection and GvHD prophylaxis type ( $p = 0.314$ ).

Overall mortality during the first year after HSCT in patients with HAdV infection was comparable to that without HAdV infection: 20.3 vs. 20.8 %;  $p = 0.954$  (Fig. 1) and measured cumulative incidence in the same groups was 0.203 vs. 0.208, respectively (Fig. 2). Thirteen of 64 patients with detected HAdV DNAemia died, in comparison to 10 from 48 persons without detectable HAdV DNA. The median survival for all patients with HAdV infection was 117 days. Of those 13 patients, four persons died during ongoing viraemia of HAdV aetiology. Patients from this group showed median HAdV viral load in blood comparable to patients who died at the time when there was no HAdV DNAemia ( $p = 0.812$ ). In two patients, sepsis was the direct cause of death, accompanied by acute respiratory distress syndrome in one of them, the other two patients died because of qPCR confirmed cytomegalovirus pneumonia.

**Table 5** Comparison of variables between HAdV-positive and HAdV-negative patients

	Patients with adenoviraemia <i>N</i> = 64	Patients without adenoviraemia <i>N</i> = 48	<i>p</i> value
Median age in years (range)	40 (18–63)	43 (18–65)	<b>0.022</b>
Gender			
Male	37	22	0.212
Female	27	26	
Primary underlying disease			
Acute myeloid leukemia	29	24	0.103
Other	35	24	
Stem cell donor			
Unrelated	40	26	0.263
Related	24	22	
Conditioning regimen			
Myeloablative	46	30	0.294
Non-myeloablative	18	18	
GvHD	18	5	<b>0.018</b>
Anti-GvHD prophylaxis			
Cyclosporine + methotrexate	47	31	0.314
Cyclosporine + mycophenolate mofetil	17	17	
Cumulative incidence of death (95 % CI)	0.203 (0.098–0.296)	0.208 (0.085–0.315)	0.954

Statistically significant differences are in bold

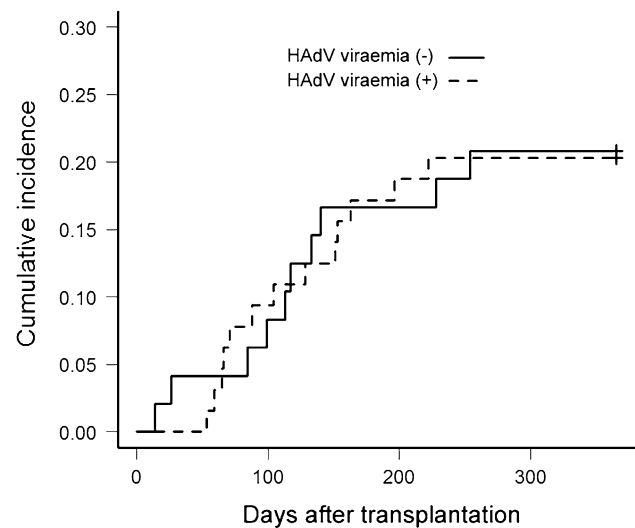


**Fig. 1** Overall mortality during the first year after HSCT in patients with HAdV infection (*solid line*) and without HAdV infection (*dashed line*).  $p = 0.954$

## Discussion

The role of HAdV infections in the morbidity and mortality of immunocompromised individuals is being increasingly recognized (Kojaoghlanian et al. 2003). Possible ways of infection spreading include primary respiratory route, faecal-oral transmission or reactivation of the virus persistent in the body. Using qPCR assay we found that 37 % of studied patients had HAdV DNA in more than one serum sample post alloPBSCT. This value is higher than reported in our previous study (19.3 %) (Rynans et al. 2012), but similar to described by other authors (32.2–44.8 %) (Bil-Lula et al. 2010; Ganzenmueller et al. 2011). Single positive results could be considered less important from clinical point of view due to possible persistent (latent) HAdV infection has been shown to occur even in healthy individuals (Alkhalaf et al. 2013). The majority of alloHSCT recipients in our study with detectable DNAemia within the first 10 weeks after transplantation had only FUO. Thirteen of 64 patients (20 %) with detected HAdV DNAemia died during first year after HSCT, which is comparable to reports of other authors (Ganzenmueller et al. 2011; Kampmann et al. 2005). In fact, HAdV-related mortality might be underestimated, due to retrospective manner of our study.

Analysis of risk factors associated with HAdV DNAemia post-alloHSCT in this study has demonstrated correlation only with occurrence of GvHD and patient age.



**Fig. 2** Cumulative incidence of death during the first year after HSCT in patients without HAdV viraemia (*solid line*) and with HAdV viraemia (*dashed line*).  $p = 0.954$

Other risk factors, including graft sources, primary disease, conditioning regimen, and anti-GvHD prophylaxis were also examined, but we did not find any of these to be associated with the development of HAdV disease. The relationship between HAdV and GvHD still remains unclear; as both may coexist and the viral infection may also be a trigger for developing of GvHD. From other side, immunosuppression associated with GvHD and its prophylaxis may increase probability of symptomatic HAdV disease. Moderate to severe GvHD (grade II–IV) has been recognized by several investigators as a risk factor for HAdV infection (Bruno et al. 2003; Runde et al. 2001). In our study patients developed only mild or moderate GvHD (grade I–II), but patients with GvHD showed significantly higher median viral load, compared to patients without GvHD. Importantly, HAdV infection may also cause diagnostic confusion, particularly mimicking gastrointestinal form of GvHD, when diarrhea is the most common presentation of those symptoms. Damage of intestinal mucosa resulting from GvHD likely increases the infectivity of HAdV leading to more severe disease in these patients. Some studies have shown that immunosuppression is directly associated with an adverse outcome of HAdV infection and recommended withdrawal or reduction of immunosuppressive treatment in patients with proven HAdV infection (Kampmann et al. 2005). Similar to previous studies (Lee et al. 2013; Robin et al. 2007), younger patients, in particular between 26 and 35 years of age, were at higher risk for HAdV infection.

In summary, we detected HAdV DNAemia in approximately one-third of adult recipients of alloPBSCT. We

conclude that used qPCR assay is a rapid and sensitive tool for detection and quantification of HAdV DNA in serum of patients undergoing alloHSCT. It could be important from the clinical point of view, as the proper diagnosis of HAdV disease in patients after alloHSCT may be delayed due to confounding picture with GvHD as discussed above, and/or possible viral co-infections. Although some patients from studied group showed signs and symptoms possibly associated to HAdV infection, no one was treated for HAdV. We cannot with this study design determine whether screening is of benefit for the patients. The introduction of PCR-based surveillance of HAdV infection has created an opportunity for early detection and pre-emptive therapy before the onset of fulminant disease and may also have predictive value for disseminated HAdV disease. Common application of molecular methods in clinical screening of HAdV DNAemia could therefore contribute to an improvement of the outcome of HAdV infections in persons subjected to alloHSCT.

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## References

- Alkhalaf MA, Guiver M, Cooper RJ (2013) Prevalence and quantitation of adenovirus DNA from human tonsil and adenoid tissues. *J Med Virol* 85:1947–1954
- Baldwin A, Kingman H, Darville M et al (2000) Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. *Bone Marrow Transpl* 26:1333–1338
- Bil-Lula I, Ussowicz M, Rybka B et al (2010) PCR diagnostics and monitoring of adenoviral infections in hematopoietic stem cell transplantation recipients. *Arch Virol* 155:2007–2015
- Bruno B, Gooley T, Hackman R et al (2003) Adenovirus infection in hematopoietic stem cell transplantation: effect of ganciclovir and impact on survival. *Biol Blood Marrow Transpl* 9:341–352
- Fischer SA (2008) Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. *Transplantation* 86:1327–1339
- Genzenmueller T, Buchholz S, Harstea G et al (2011) High lethality of human adenovirus disease in adult allogeneic stem cell transplant recipients with high adenoviral blood load. *J Clin Virol* 52:55–59
- Kampmann B, Cubitt D, Walls T et al (2005) Improved outcome for children with disseminated adenoviral infection following allogeneic stem cell transplantation. *Br J Haematol* 130:595–603
- Kojaoghlianian T, Flomenberg P, Horwitz MS (2003) The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 13:155–171
- Lankester AC (2007) Diagnosis and treatment of human adenovirus infection following allogeneic stem cell transplantation. *Rep Pract Oncol Radiother* 12:167–169
- Lee YJ, Chung D, Xiao K et al (2013) Adenovirus viremia and disease: comparison of T cell-depleted and conventional hematopoietic stem cell transplantation recipients from a single institution. *Biol Blood Marrow Transpl* 19:387–392
- Lenaerts L, Naesens L (2006) Antiviral therapy for adenovirus infections. *Antiviral Res* 71:172–180
- Lion T, Baumgartinger R, Watzinger F et al (2003) Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood* 102:1114–1120
- Lion T, Kosulin K, Landlinger C et al (2010) Monitoring of adenovirus load in stool by real-time PCR permits early detection of impending invasive infection in patients after allogeneic stem cell transplantation. *Leukemia* 24:706–714
- Matthes-Martin S, Feuchtinger T, Shaw PJ et al (2012) European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transpl Infect Dis* 14:555–563
- Öhrmalm L, Lindblom A, Omar H et al (2011) Evaluation of a surveillance strategy for early detection of adenovirus by PCR of peripheral blood in hematopoietic SCT recipients: incidence and outcome. *Bone Marrow Transpl* 46:267–472
- Robin M, Marque-Juillet S, Scieux C et al (2007) Disseminated adenovirus infections after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcome. *Haematologica* 92:1254–1257
- Rola A, Przybylski M, Dzieciatkowski T et al (2007) Detection of human adenoviruses with real-time PCR assay using TaqMan fluorescent probes. *Med Dosw Mikrobiol* 59:371–377
- Runde V, Ross S, Trensche R et al (2001) Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multicenter surveillance study. *Bone Marrow Transpl* 28:51–57
- Rynans S, Dzieciatkowski T, Basak GW et al (2012) Human adenovirus infection in patients subjected to allogeneic hematopoietic stem cell transplantation—a three-year single center study. *Acta Virol* 56:85–87
- Sive JI, Thomson KJ, Morris EC et al (2012) Adenoviremia has limited clinical impact in the majority of patients following alemtuzumab-based allogeneic stem cell transplantation in adults. *Clin Infect Dis* 55:1362–1370
- Suparno C, Milligan DW, Moss PA et al (2004) Adenovirus infections in stem cell transplant recipients: recent developments in understanding of pathogenesis, diagnosis and management. *Leuk Lymphoma* 45:873–885
- Taniguchi K, Yoshihara S, Tamaki H et al (2012) Incidence and treatment strategy for disseminated adenovirus disease after haploidentical stem cell transplantation. *Ann Hematol* 91:1305–1312