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Viral infections

Adenoviral infection after allogeneic stem cell transplantation (SCT): report on 130 patients from a single SCT unit involved in a prospective multi center surveillance study

V Runde[1], S Ross[2], R Trenschel[1], E Lagemann[1], O Basu[3], K Renzing-Köhler[4], UW Schaefer[1], M Roggendorf[2] and E Holler[5]

[1]*Department of Bone Marrow Transplantation,* [2]*Institute of Virology,* [3]*Department of Pediatric Hematology and Oncology,* [4]*Institute for Medical Informatics, Biometry and Epidemiology, University Hospital Essen; and* [5]*Department of Hematology and Oncology, University Hospital Regensburg, Germany*

Summary:

The incidence of adenovirus (AV) infections following SCT was determined in a prospective multicenter trial. Over 1 year, 130 consecutive patients undergoing allogeneic SCT at Essen University Hospital were included and followed for 6 months. Source of stem cells was blood in 68 cases. Fifty-eight patients had HLA-identical sibling donors. Throat swabs, urine and stool samples were screened weekly for AV antigen and DNA by ELISA and nested PCR, respectively. In 35 cases adenovirus infection was detected. There was no seasonal variation. Throat swabs were positive in 24, urine in 12, and stool in 11 cases, resulting in a cumulative risk of infection of 29%. The incidences of AV infection of the respiratory, gastrointestinal and urinary tract were 19%, 10%, and 9%, respectively, and infections were diagnosed after a median (range) interval of 44 (–2–179), 37 (–2–168), and 53 (17–153) days after transplantation. On multivariate analysis, presence of AV antibody in the donor and acute graft-versus-host disease grade IV were found to be independent risk factors for AV infection. Eleven patients had AV isolated from more than one site and five patients had probable AV disease. We were not able to identify patients in whom AV infection was the leading cause of death. The majority of patients infected with AV suffered from severe acute graft-versus-host disease often accompanied by other opportunistic infections, such as aspergillosis or CMV reactivation. Nineteen out of 36 patients who died during the observation period had AV infection. In summary, AV infection after allogeneic SCT was observed in a substantial number of patients. In addition to well-known risk factors for viral infection after SCT we were able to demonstrate that a positive AV antibody test in the donor is an important risk factor for AV infection. Further studies are needed, however, before final conclusions on the clinical sequelae of AV infection can be made and the role of preventive and therapeutic strategies toward AV infection after allogeneic SCT can be defined. *Bone Marrow Transplantation* (2001) **28,** 51–57.

Keywords: adenoviral infection; allogeneic stem cell transplantation; prospective surveillance study

Hemopoietic stem cell transplantation has been used with increasing success for treatment of patients with various hematological neoplasms, but the procedure itself is associated with significant morbidity and mortality. Infectious complications are major factors contributing to transplant-related mortality. In the past, the most common viral infections following marrow transplantation were caused by herpes simplex virus (HSV), cytomegalovirus (CMV), and varicella zoster virus (VZV). With the introduction of acyclovir for HSV and VZV and ganciclovir treatment for CMV, fatalities caused by these viruses have decreased dramatically. Over the same period of time, reports on fatal viral infections caused by adenovirus (AV) have been published by several groups.[1–3] The most common clinical manifestation of AV infection is hemorrhagic cystitis,[4] followed by gastroenteritis, pneumonitis, and liver failure.[5] In addition, AV can cause pancreatitis, nephritis, and disseminated disease leading to mortality rates ranging from 18 to 60%. The outcome of AV disease depends on patient age, type of immunosuppression, and AV serotype.[6] An increasing incidence of AV infection has been observed in recipients of T cell-depleted allogeneic marrow transplants,[7,8] in patients with donors other than HLA-identical siblings and in patients receiving total body irradiation (TBI) containing conditioning regimens.[9]

The primary objective of our study was to prospectively analyze the incidence of AV infection and AV disease over 1 year. Secondary objectives were to determine the seasonal frequency and the risk factors for AV infection and disease and to study the clinical sequelae of AV infection.

Correspondence: Dr V Runde, Department of Bone Marrow Transplantation, University Hospital, Hufelandstrasse 55, 45122 Essen, Germany
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Patients and methods

One hundred and thirty consecutive patients undergoing allogeneic SCT at the Department of Bone Marrow Transplantation and the Department of Pediatric Hematology and Oncology were studied. Pretransplant conditioning, choice of both the stem cell source and the donor, and prophylaxis of acute GVHD were performed according to disease-specific protocols (Table 1). Strict reverse isolation with a high efficiency particulate air filtration system and standard decontamination procedures was performed as previously reported.[10] Patients were included between 1 February 1998 and 31 January 1999 and were followed until day 180 post transplant.

Prior to admission for SCT, the donor and recipient were serologically screened for antibodies (IgG) against AV. Screening for AV DNA in peripheral blood mononuclear cells was not performed. Additionally, a swab and urine sample from the recipient were screened by antigen ELISA. Between admission and discharge, appropriate specimens were taken weekly. The first specimens were collected prior to pretransplant conditioning. For surveillance, throat swabs and urine samples were mandatory. These samples were tested by antigen ELISA. After discharge appropriate specimens were taken at least twice before day 100 (for example between day 50–60 and 80–120) and once until day 180 (day 150–180).

In patients with respiratory symptoms, significant diarrhea or hemorrhagic cystitis additional analysis of nasopharyngeal wash, aspirate or throat swabs, sputum and, if possible, bronchoalveolar lavage (atypical pneumonia, interstitial pneumonia, severe bronchitis), stool (diarrhea) and urine (cystitis) were performed at the time of clinical diagnosis. Inclusion of diagnostic procedures for CMV was mandatory in all patients and for all samples obtained.

Viral specimens were taken from all patients showing the symptoms indicated independent of clinical judgement, because AV infection may be the cause of these complications and gastrointestinal symptoms as well as hepatic involvement need not necessarily be the result of acute GVHD or other toxicities of SCT such as veno-occlusive disease. Specimens were collected for each patient and analyses were performed at the end of the surveillance period.

Table 1 Patient and transplant characteristics

Age range (median) years	1–59 (37)
Male	79
Female	51
Diagnosis	
CML	59
AML	36
ALL	11
MDS	10
Aplastic anemia	5
Lymphoma	3
Others	6
Conditioning	
ATG containing	26
TBI without ATG	90
Chemoconditioning without ATG	14
Type of transplant	
Bone marrow	62
Peripheral stem cells	62
CD34 enriched peripheral stem cells	6
Type of donor	
HLA identical sibling	58
Alternative family donor	22
Matched unrelated donor	50
AGVHD	
Grade I	47
Grade II	30
Grade III	12
Grade IV	14
CMV	
Seropositive recipients	70
pp65 reactivation	57
Patient outcome	
Death after relapse	4
Death without relapse	35

Detection of AV antigen

Adenovirus antigen was detected in throat swabs by a direct immunofluorescence assay (Light Diagnostics, Temecula, CA, USA) and in urine and stool samples by Elisa (RIDASCREEN, R-Biopharm, Darmstadt, Germany) according to the manufacturer's instructions.

Detection of AV DNA by PCR

DNA was extracted with Viral DNA kit (Qiagen, Hilden, Germany). To check for a possible inhibitory effect on PCR of substances present in the urine and stool, specimens were spiked with adenovirus type 2 and type 5 positive cell culture supernatants at a final concentration of 1:100. Adenovirus DNA could be invariably detected in these controls. For nested PCR, primers derived from the well-conserved hexon gene sequence were used: HexA 5′-GCC GCA GTG GTC TTA CAT GCA CAT C-3′ (PCR 1, nt 18858 to 18882, numbering according to human adenovirus type 2 GenBank sequence J01917); HexB 5′-CAG CAC GCC GCG GAT GTC AAA GT-3′ (PCR 1, nt 19136 to 19158); ADP-1 5′-CCA GGA CGC CTC GGA GTA CCT-3′ (PCR 2, nt 18888 to 18908), and ADP-2 5′-GGA AGC CAT ATC AAG CAC AC-3′ (PCR 2, nt 19112 to 19131). These primers could detect all adenovirus types. Amplified DNA from the second PCR was fractionated by 1% agarose gel electrophoresis and the 243-bp fragment was visualized by UV fluorescence after staining with ethidium bromide.

Virus isolation

Throat swabs, urine, and suspensions of stools were centrifuged (4°C, 30 min, 3000 r.p.m.) and the supernatant was passed through 0.45 μm filters. The filtered supernatant was used to inoculate duplicate roller tubes containing confluent monolayers of human (foreskin) fibroblasts and monkey kidney cells. After 1 h of adsorption at 37°C, minimal essential medium supplemented with 2% fetal calf serum was added. Cultures were then incubated at 37°C in a roller drum and were examined for cytopathic effects daily for at least 1 week.

Detection of AV antibodies

Antibodies were determined by a commercially available assay (Elisa Classic Adenovirus Ig G, Virion, Würzburg, Germany) according to the manufacturer's instructions.

CMV antigenemia assay

The CMV antigenemia test was performed as described in full detail elsewhere.[11]

Clinical parameters

Besides the demographic data shown in Table 1, the following items were recorded and evaluated: stage of disease, immunosuppressive prophylaxis, immunosuppressive treatment (overall and at the time of diagnosis of AV infection), day of onset of acute GVHD, day of onset of CMV reactivation, occurrence of respiratory and gastrointestinal symptoms and of hemorrhagic cystitis, day of onset of symptoms, and other infections occurring after transplantation.

Definition of AV infection and disease

Diagnosis of AV infection was based on the detection of viral DNA in at least one specimen and/or the identification of AV antigen in consecutive samples. Definitive and probable AV disease were diagnosed according to published criteria.[2] Definite disease was defined by a positive tissue culture from a sterile site (excluding gastrointestinal tract), the presence of typical AV nuclear inclusions on histopathology, or both in the presence of signs and symptoms compatible with adenoviral disease. Probable disease was defined as the presence of two or more positive tests from other body sites in association with compatible symptoms without other identifiable cause.

Treatment of viral infections

Since the aim of the study was to collect the surveillance data on AV infection, all relevant analyses were performed at the end of the study period. Therefore, information on AV infection was not available to the physicians during hospitalization of the patients and, thus, no specific treatment against AV was initiated. Treatment of other viral infections during the surveillance period was decided by treating physician. Prophylactic immunoglobulin infusions or prophylactic antiviral treatment were not given. With occurrence of mucositis, typical herpetiform skin lesions, or isolation of HSV in throat swabs intravenous acyclovir treatment was initiated. Pre-emptive treatment with ganciclovir was started when pp65 antigenemia was detected.

Statistical evaluation

Initial exploratory analysis was performed using the chi-square test or Fischer's exact test (for small expected frequencies) to compare proportions of events between subgroups. For time dependent events, the Kaplan–Meier method was used. The log-rank test was applied to compare subgroups. Cox proportional hazard regression model was performed adjusting for effects that turned out to be significant in univariate analysis. A stepwise variable selection procedure was used with a cut-off level at 0.05 for inclusion of values which had proven to be significant in the initial exploratory model. Analysis was performed using the SPSS statistical program for Windows, version 8.0.

Results

AV status of recipient and donors before SC

Sixty-six patients (51%) were AV IgG-positive. Of 119 donors with known AV antibody status 35 individuals (29%) were AV IgG-positive. No difference between younger and older patients with regard to AV antibody status of recipients or donors could be demonstrated. Univariate analysis showed a positive correlation between a positve AV antibody test in the donor or recipient and a more advanced stage of the underlying disease and acute graft-versus-host disease (aGVHD).

AV excretion

In 35 patients, AV DNA in at least one specimen and/or the identification of AV antigen in consecutive samples from the same patient was detected (Table 2). The cumulative risk of infection within 6 months was 29%. Median time from transplant to first AV detection in specimens from the respiratory tract was 44 days (range, –2–179), in urine samples 37 days (range, –2–168), and in stools 53 days (range, 17–153). Cumulative risks within 6 months for AV excretion in sputum, urine, and stool were 19%, 10%, and 9%, respectively. In 13 patients viral excretion was only found in samples from the respiratory tract, in three patients only in urine, and in seven patients only in stools. The other patients tested positive for AV had findings in two or even all three sites. There was no seasonal variation in AV infection (Figure 1).

On univariate analysis (log-rank) AV infection was associated with positive AV antibody status of the donor ($P < 0.01$), older age of the patient ($P = 0.03$), use of unmanipulated peripheral stem cells ($P = 0.01$), more advanced stage of disease ($P = 0.01$), grade IV aGVHD ($P < 0.001$), use of anti-lymphocyte globulin (ALG) in conditioning regimens ($P = 0.03$), and use of alternative family donors ($P = 0.01$). AV antibody status of the recipient reached no significance ($P = 0.08$). CMV antibody status of the recipient and CMV reactivation were not associated with AV infection.

Recipients with negative AV antibody tests had a cumulative risk of 50% when the donor was positive for AV antibodies and of 12% when the donor was negative for AV antibodies (Figure 2). Recipients with positive AV antibody tests having donors with negative AV antibody tests had a cumulative risk of 32% to develop AV (Figure 3). Patients with PBSC had a 39% risk of AV infection, whereas patients after BMT or CD34 enriched SCT had only a cumulative risk of 19% (Figure 4). Patients with ALG conditioning had a significant earlier onset of AV infection (Figure 5). The risk of patients with alternative family

Table 2 Patients with adenoviral excretion

Patients No.	*Adenoviral AB status*		*Onset of symptoms or adenoviral excretion (days after SCT)*						*Death (day)*
	Pat.	*Don.*	*Respiratory*		*Urine*		*Stool*		
			Sympt.	*AV*	*Sympt.*	*AV*	*Sympt.*	*AV*	
4	1	1					25	27	41
6	1	0	28				60	53	121
12	1	0	91	168	166	168	47	153	
20	0	1	56				41	56	
23	1	0	32	30					179
26	1	0	4	–2					10
29	1	1	116	113		132			136
36	1	1	118				27	37	95
41	1	0	70			–2	35		94
45	0	0			24	30			
51	0	0	175	179	83	71			
52	0	1		63					
56	0	1	7	4					10
64	0	0	169	177		14			
66	0	1	25	22	17				33
80	1	?		29					
82	1	0				133			
88	0	1	40	80	54	51			170
99	1	0	10	63	13		58	72	108
102	1	1	33	77			36	63	80
104	1	1	94	109					
106	1	0	37	44	30	44			63
112	1	1	46	103			11	17	173
114	1	0				20	29		65
118	0	1		20		20			
125	0	0		16					
129	0	0	19	29					82
136	1	1	70				74	76	
142	1	1	29	44					60
145	1	?	10	13	10	13			
147	0	1		23					
150	1	0					23	36	
151	1	1	28	35					69
155	1	0	15				10	20	54
157	1	0		14					

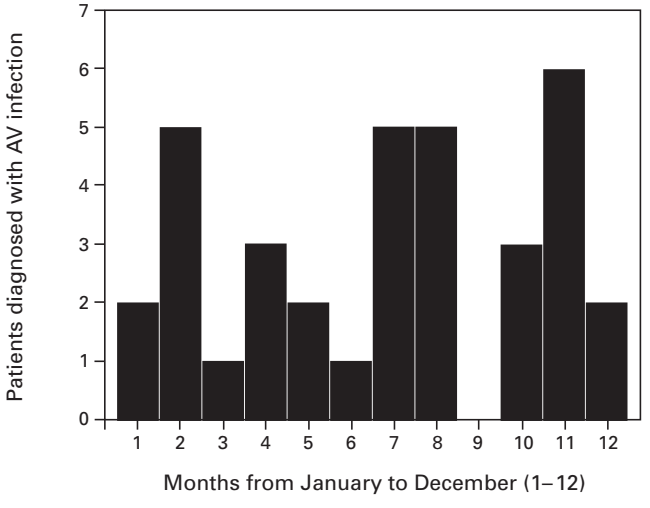

Figure 1 Distribution of onset of adenoviral infection in 35 patients from January to December (1–12) during the year of study.

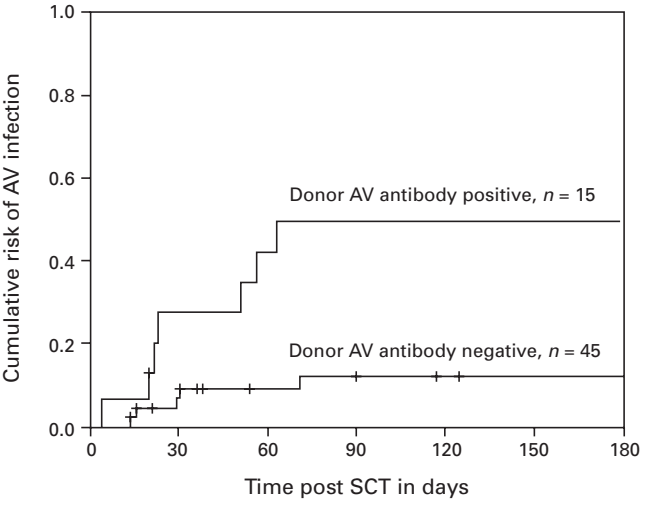

Figure 2 Cumulative risk of AV infection in patients with negative AV antibody tests depending on the AV antibody status of the donor ($P = 0.001$). Donor AV antibody negative, $n = 45$; donor AV antibody positive, $n = 15$.

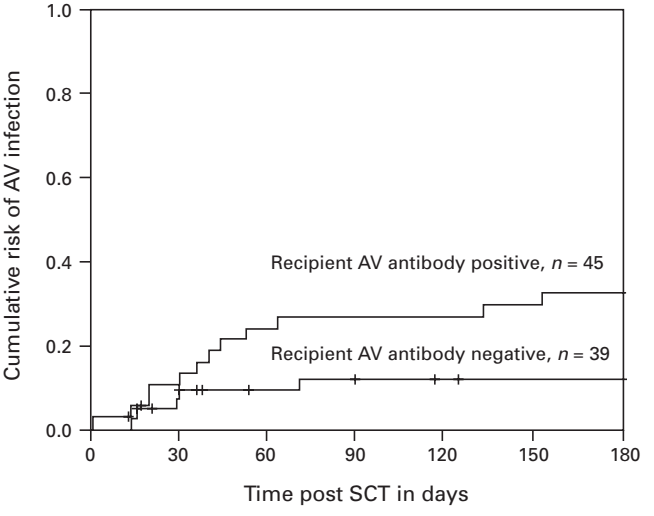

Figure 3 Cumulative risk of AV infection in patients with negative AV antibody status of the donor depending on the recipient AV antibody status ($P = 0.08$). Recipient AV antibody negative, $n = 45$; recipient AV antibody positive, $n = 39$.

donors to develop AV infection was 49% compared to only 24% in patients with other donors (Figure 6).

Multivariate analysis in a Cox model included these predictors: stage of disease at SCT, source of stem cells (unmanipulated PBSC *vs* BM, CD34), conditioning with ATG, use of an alternative family donor, grade IV aGVHD, AV antibody status of donor, AV antibody status of the recipient, and patient's age. Significant positive multivariate associations between AV infection were found for (1) positive AV antibody status of the donor ($P = 0.01$; RR 2.5) and (2) grade IV aGVHD ($P = 0.048$; RR 3.1). A weak positive association was found for (3) age above 37 years ($P = 0.06$; RR 2.12) and (4) use of unmanipulated peripheral SCT ($P = 0.07$; RR 2.02).

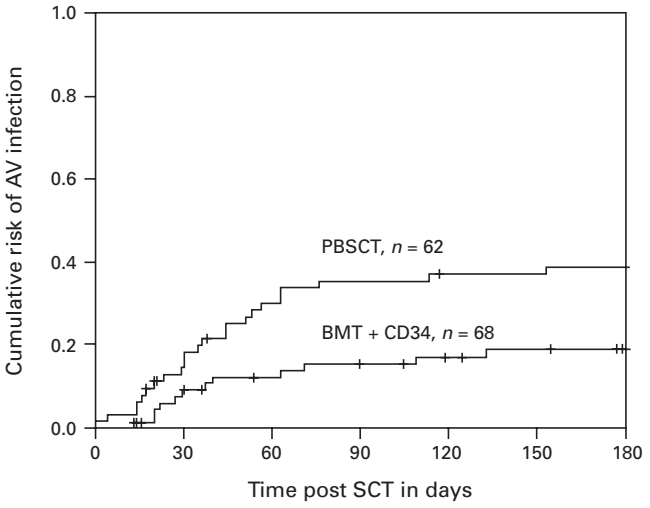

Figure 4 Cumulative risk of AV infection after unmanipulated peripheral blood stem cell transplantation (PBSCT) compared to bone marrow (BM) and CD34-enriched PBSCT (CD34) ($P = 0.01$) PBSCT, $n = 62$; BMT + CD34, $n = 68$.

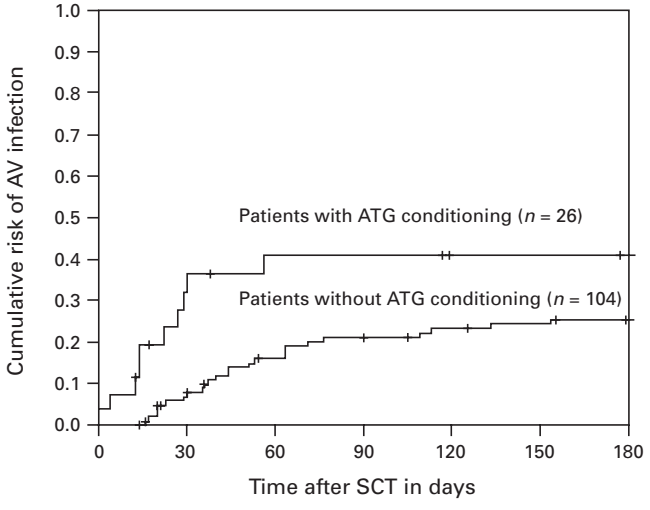

Figure 5 Cumulative risk of AV infection after conditioning with ATG ($n = 26$) and without ATG ($n = 104$) ($P = 0.03$).

Clinical manifestation and AV disease

Eighteen of 24 patients with respiratory AV excretion had respiratory symptoms. Another 25 patients had respiratory symptoms without respiratory AV excretion after a median interval of 25 days after transplantation (range, day 7–170). Six of 12 patients with AV detection in the urine had hematuria. Another 10 patients developed hematuria at a median interval of 20 days (range, day 13–117) and developed no AV excretion. According to the study protocol, only patients with diarrhea were screened. Therefore, no information about asymptomatic AV carriers of the gastrointestinal tract is available. Eleven of 18 patients with significant diarrhea had AV present in the stool.

Eight patients had two or more positive AV tests from other body sites associated with compatible symptoms. Often, there were overlaps between the clinical manifestations of AV infections and other conditions, such as aGVHD or other infectious diseases. This was particularly true for patients from whom AV was isolated from the gastrointestinal tract and who had bloody diarrhea. Four patients with fecal AV excretion had diarrhea that could be explained clinically by the occurrence of aGVHD. In three patients, besides AV infections with aspergillus fumigatus, CMV, and poliomavirus BK were found, respectively. These co-infections could have also caused the respiratory symptoms or the cystitis.

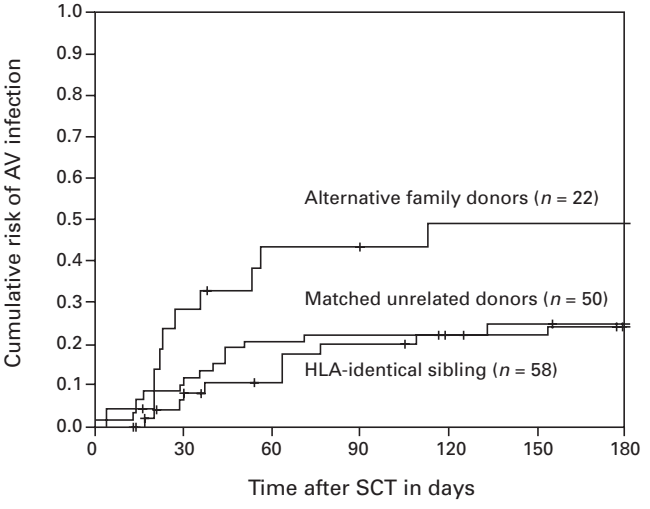

Figure 6 Cumulative risk of infection after SCT with an HLA-identical sibling ($n = 58$), alternative family donors ($n = 22$), and matched unrelated donors ($n = 50$) ($P = 0.01$).

According to the definition of Flomenberg *et al*,[2] five of 35 patients with AV excretion had a probable AV disease. Biopsies during the early post-transplant phase were only performed in a minority of patients and revealed no finding consistent with definite AV disease. Autopsies in three of five patients that presented two or more positive tests failed to show histopathologic confirmation of invasive disease at the time of death.

Mortality and AV infection

Nineteen of 35 patients (54%) with AV infection died during the observation period. In 11 cases autopsy was performed. In none of these patients was AV disease identified as the leading cause of death. Two of these patients died from relapse of their malignant disease. One died from veno-occlusive disease of the liver. The other patients died from severe aGVHD or opportunistic infection associated with GVHD-treatment: lethal infections were aspergillus pneumonia or generalized aspergillus infection in six cases, CMV pneumonia in two cases, toxoplasmosis in one case, candida septicemia in one case, methicillin-resistant *Staphylococcus aureus* septicemia in one case, and EBV-associated lymphoproliferative disease in one case, respectively. Two patients with severe aGVHD (No. 4 and No. 114) died from multi-organ failure after development of a systemic inflammatory syndrome without identifiable infectious agent. One patient died from multi-organ failure after development of hyperacute GVHD (No. 56). One patient

(No. 88) had multiple infectious complications and developed cerebral lesions that were not caused by an infectious agent. We could identify no patient who died from the sequellae of AV infection. Of 95 patients without AV infection, 16 patients (19%) died during the observation period. This was a significantly lower risk than in patients with AV infection.

Discussion

In this study, AV infection was diagnosed when viral DNA was detectable in one specimen and/or AV antigen was identified in consecutive samples from the same patient. Based on this definition, the cumulative incidence of AV infection during the first 6 months following allogeneic SCT was 29%. This was substantially higher compared to other studies reporting incidences varying from 4.9% to 20.9%.[1,2,7,12–15] The reported figures from these studies, however, might represent an underestimate, because incidence rates are influenced by policies regarding the timing and indications for obtaining cultures, as well as culture techniques used. The higher incidence in our study may, at least partly, have occurred because we obtained weekly samples from the upper respiratory tract and urine even in asymptomatic patients. Nine of 35 patients in whom AV infection was detected were asymptomatic. This would have resulted in a 20% incidence if we had performed a symptom-directed culturing strategy as reported by others.[15] In conclusion, it can be speculated, that about one third of patients with AV infections after allogeneic SCT remain asymptomatic and AV is only an 'innocent bystander' in these cases. From this observation it can not be concluded that the other two thirds of patients developed symptoms which were caused by the AV identified. In our series, four of 26 symptomatic patients excreting AV in the stool had diarrhea which started in the clinical context of severe aGVHD. In addition, in three other symptomatic patients with AV excretion other infectious agents were found that could explain their symptoms. Thus, only 19 of 130 patients (15%) developed symptoms that could be solely attributed to AV infection.

Our study shows that AV antibody status of the donor has a strong, independent influence on the development of AV infection in the recipient. This finding seems to support the hypothesis that AV infections after SCT are not always reactivations of latent viruses in the recipient but may also arrive by 'transfusion' of cells from AV antibody-positive donors. To sustain this hypothesis, molecular analyses are underway to determine whether or not donors and corresponding recipients really are infected with the same AV isolate.

In contrast to CMV, where CMV antibody status of the donor has only a minor impact on CMV reactivation,[11] AV antibody status of the donor had a strong impact on the development of AV infection in the recipient on both univariate and multivariate analysis. This is in line with the notion that the use of unmanipulated stem cells (PBSC) has been identified as a factor that increases the risk for development of AV infection in the recipient. PBSC transplants contain 10 to 20 times more lymphocytes compared to bone marrow which may, therefore, account for the increased risk of AV transmission after PBSC. We recently published data on CMV infections comparing PBSC and BM SCT and found similar results for CMV seronegative recipients with CMV positive donors.[11] Published data concerning the presence of adenovirus DNA in peripheral blood mononuclear cells from healthy adults are, however, controversial but there is experimental evidence that these cells might contain AV DNA. Therefore, AV DNA from the donors' PBMCs could be the source of AV infection in the recipient after SCT. Our findings on the strong impact of donor AV status indirectly support this hypothesis. However, further studies have to unequivocally demonstrate whether or not donors and recipients really are infected with the same AV isolate. Horvath *et al*[16] detected AV DNA in peripheral lymphocytes in 13 of 17 healthy adults, whereas Flomenberg *et al*[17] could not detect AV DNA in mononuclear cells of 33 adults. A transmission of AV through transfusions given after SCT seems to be very unlikely since our patients only received leukocyte-filtered blood components. Transmission of AV from visitors or from staff can not be excluded with certainty, but it should be stressed that all patients were treated under strict protective measures including use of masks and air filtration. In addition, no seasonal effect of AV infections and no clusters were identified.

The second independent risk factor was the presence of acute GVHD grade IV. This finding agrees with studies from Hale *et al*,[9] who found that GVHD was a risk factor for AV infection on univariate analysis and Flomenberg *et al*[2] who reported that moderate to severe aGVHD was an independent risk factor for AV disease. These findings were not unexpected because acute GVHD itself and the necessary treatment cause severe immunodeficiency. From other viral infections following SCT, such as CMV, it is also well known that aGVHD is an independent risk factor for reactivation.[11] In this study, use of stem cells from not completely HLA-matched family donors was found to be another risk factor for AV infection in addition to acute GVHD. One might conclude, therefore, that in general patients with a compromised immune system due to GVHD or immunosuppressive treatment become more susceptible to a wide variety of infections including AV.

In contrast to other studies reporting a higher risk of AV infection in children, we demonstrated that the risk of AV infection rises with increasing age.[2,15] An explanation for our finding might be that only a minority of our patients (11%) were below the age of 16 years and, therefore, a possibly higher risk of AV infection in children was not identifiable in our patient group. The fact that the age-related risk of AV infection is independent from the risk factor GVHD is not surprising: older patients have a significantly higher risk of transplant-related mortality which is at least partly attributable to a more pronounced susceptibility to opportunistic infections.

Eleven patients in our cohort (31%) had AV isolated from more than one site. This proportion is within the range of values reported by Shields *et al*[1] and Howard *et al*[15] who found AV in more than one site in 19% and 39% of their infected patients. According to the definition of Flomenberg

et al,[2] five patients with AV infection had probable AV disease.

Nineteen of 35 patients (54%) with AV infection died during the observation period which represents a significantly higher 6-month mortality rate than was seen in patients without AV infection (19%). Death of patients with AV infection was mainly attributed to overwhelming infections following acute GVHD, such as aspergillus or CMV pneumonia. In some of these cases, significant impact from AV was suspected, but autopsy failed to identify patients with definite AV disease (ie positive tissue culture from a sterile site or the presence of typical adenoviral nuclear inclusions on histopathology). This finding was in contrast to the study of Howard *et al*[15] who stated that 11 deaths (2%) within their cohort of patients were attributable to AV alone. A possible explanation for our finding is that we performed autopsies in only three of five patients with AV in more than one site.

We conclude that patients with AV infection after allogeneic SCT are often seriously ill and have a much higher mortality rate than do patients who do not develop AV infections. These patients develop multiple opportunistic co-infections in the context of severe aGVHD and the contribution towards morbidity caused by AV infection is difficult to determine, even within the prospective design of our surveillance study. In our series we were not able to identify patients in whom AV infection was the leading cause of death. In a larger retrospective series, the incidence of lethal AV infections was 2% which confirms that death caused by AV after allogeneic SCT seems to be a rare event.[15] Future studies should investigate the role of prophylactic, pre-emptive and therapeutic strategies for AV infection after allogeneic SCT.

References

1 Shields AF, Hackman RC, Fife KH *et al.* Adenovirus infections in patients undergoing bone marrow transplantation. *New Engl J Med* 1985; **312**: 529–533.
2 Flomenberg P, Babbitt J, Drobyski WR *et al.* Increasing incidence of adenovirus disease in bone marrow transplant recipients. *J Infect Dis* 1994; **169**: 775–781.
3 Matsuse T, Matsui H, Shu C-Y *et al*. Adenovirus pulmonary infection identified by PCR and *in situ* hybridisation in bone marrow transplant recipients. *J Clin Pathol* 1994; **47**: 973–977.
4 Ambinder RF, Burns W, Forman M *et al.* Hemorrhagic cystitis associated with adenovirus infection in bone marrow transplantation. *Arch Intern Med* 1986; **146**: 1400–1401.
5 Bertheau P, Parquet N, Ferchal F *et al.* Fulminant adenovirus hepatitis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1996; **17**: 295–298.
6 Hierholzer JC. Adenoviruses in the immunocompromised host. *Clin Microbiol Rev* 1992; **5**: 262–274.
7 Blanke C, Clark C, Broun R *et al.* Evolving pathogens in allogeneic bone marrow transplantation: increased fatal adenoviral infections. *Am J Med* 1995; **99**: 326–328.
8 Childs R, Sanchez C, Engler H *et al.* High incidence of adeno- and polyomavirus-induced hemorrhagic cystitis in bone marrow allotransplantation for hematological malignancy following T cell depletion and cyclosporine. *Bone Marrow Transplant* 1998; **22**: 889–893.
9 Hale GA, Heslop HE, Krance RA *et al.* Adenovirus infection after pediatric bone marrow transplantation. *Bone Marrow Transplant* 1999; **23**: 277–282.
10 Beelen DW, Haralambie E, Brandt H *et al.* Evidence that sustained growths suppression of intestinal anaerobic bacteria reduces the risk of acute graft-versus-host disease after sibling bone marrow transplantation. *Blood* 1992; **80**: 2668–2676.
11 Trenschel R, Ross S, Hüsing J *et al.* Allogeneic transplantation of peripheral blood stem cells reduces the risk of persisting cytomegalovirus pp65 antigenemia and cytomegalovirus related interstitial pneumonia. *Bone Marrow Transplant* 2000; **25**: 665–672.
12 Wassermann R, August CS, Plotkin SA. Viral infections in pediatric bone marrow transplant patients. *Pediatr Infect Dis* 1988; **7**: 109–115.
13 Munoz FM, Piedra PA, Demmler GJ. Disseminated adenovirus disease in immunocompromised and immunocompetent children. *Clin Infect Dis* 1998; **27**: 1194–1200.
14 Bruno B, Boeckh M, Davis C *et al*. Adenovirus infection in patients undergoing bone marrow transplantation. *Blood* 1997; **90**: 1867a.
15 Howard DS, Phillips GL, Reece DE *et al.* Adenovirus infection in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 1999; **29**: 1494–1501.
16 Horvath J, Palkonyay L, Weber J. Group C adenovirus DNA sequences in human lymphoid cells. *J Virol* 1986; **59**: 189–192.
17 Flomenberg P, Gutierrez E, Piaskowski V, Casper JT. Detection of adenovirus DNA in peripheral blood mononuclear cells by polymerase chain reaction assay. *J Med Virol* 1997; **51**: 182–188.