Disseminated adenovirus infections after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcome

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ABSTRACT

We analyzed the factors and outcome of patients with disseminated adenovirus infection (dAdV) after allogeneic hematopoeitic stem cell transplantation (HSCT). Thirty patients with dAdV were identified among 620 allogeneic HSCT recipients. Primary diseases were leukemia (n=17), Fanconi anemia (n=12) or others (n=1). Source of stem cells was unrelated in 28 and related in 2 patients. The graft consisted of peripheral blood (n=3), bone marrow (n=12) and unrelated cord-blood (UCB, n=15). Risk factors for dAdV in unrelated HSCT recipients were previous Fanconi disease (p=0.03) and GVHD (p=0.02) in children, and cord blood source of stem cells (p=0.029) and GVHD (0.024) in adults.

Key words: adenovirus infection, allogeneic hemetopoietic stem cell transplantation, risk factors.

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Allogeneic hematopoietic stem cell transplantation (HSCT) can cure benign or malignant hematologic diseases. Unfortunately, HSCT is followed by immune deficiency, eventually worsened by graft versus host disease (GVHD) and its immunosuppressive treatment. The reported incidences of adenoviral (AdV) infection and disease vary 8-47% and have become increasingly frequent in recent years.[1-3] Progression to a disseminated disease has been suggested in approximately 10-20% of patients with probable AdV infection. The detection of an adenoDNAemia is highly predictive of disseminated disease.[4-6] The mortality rate is low in AdV infection but high in invasive disease (20-80%).[6,7] The aim of this retrospective unicentric study was to determine clinical characteristics, outcome and risk factors of disseminated AdV infections.

Design and Methods

In our center, patients with clinical signs compatible with a viral infection, have had adenovirus screening in peripheral sites and blood sice 2000. Disseminated (d) AdV infection was defined as clinical signs associated with a positive adenoDNAemia as previously described.[4-6] AdV was detected by Enzyme-Linked-Immunosorbent-Assay (ELISA) or PCR in stools and by PCR in broncho-alveolar lavage and urine. Standard AdV PCR was performed according to the published method.[8,9] Real-time AdV PCR has been performed since 2005 according to the published method.[10] Real time AdV PCR has been prospectively performed in blood plasma, once a week until the third month after transplant since 2005, and was retrospectively performed in patients who before this date had positive standard PCR. Patients who reached 10,000 copies /ml AdV in blood plasma by real time PCR were always positive by s-PCR. Real-time AdV PCR was positive in a median 11 days before s-PCR (n=18, 79 days vs. 90 days after HSCT). Among 6 patients who did not reach 10,000 copies/ml in blood plasma, 2 were positive by s-PCR, 1 was negative and 3 were not tested by s-PCR. These three patients were excluded from the statistical analysis of cumulative incidences of dAdV and risk factors to avoid bias from over diagnosis related to real time PCR sensitivity. Diarrhea, cystitis and pneumonia were attributable to AdV if AdV was found in stools, urine and broncho-alveolar lavage respectively. Hepatitis with transaminases more than 5 times the normal range was considered a possible AdV hepatitis. From January 2000 to July 2006, 30 patients

satisfied dAdV criteria among the 620 patients who underwent HSCT in our center, including 273 unrelated recipients. Treatment by cidofovir was started if patients were considered to be in a curative phase (5 mg/kg/week or 1 mg/kg x 3/week).

The probabilities of dAdV infection and adenoDNAemia clearance were calculated on the basis of their cumulative incidence, with death as competing event. The probability of overall survival (OS) was calculated according to the Kaplan-Meier method. Potential risk factors for survival and dAdV occurrence were tested using the Cox proportional hazard regression model. Potential predictors for cidofovir response were tested using Fine and Gray's test. S-PLUS 2000 Professional was used for all statistical analyses.

Results and Discussion

AdV disease in HSCT recipients is increasingly recognized as a significant cause of morbidity and mortality. From 2000 to 2006, dAdV was identified in 30 out of 620 patients. This represents one of the largest dAdV series. Two patients were diagnosed after an HLA-matched sibling transplant (an 18-year old with a Fanconi anemia and a 48-year old with acute leukemia). The other 28 patients received an unrelated transplant (see Table 1 for patient characteristics). dAdV occurred in a median 81 days (18-460) after HSCT. A late infection (> 3 months after transplantation) occurred in 13 HSCT recipients and was more frequent in children (9/15 vs. 5/15). Late onset of dAdV infection is unusual and in this context late AdV disseminated infections should be taken into consideration.

The most frequent clinical signs were diarrhea (n=24) and hepatitis (n=12) followed by pneumonia (n=7) and cystitis (n=6). Clinical signs in multiple organs were observed in 24% of patients and 80% of patients were febrile. All patients had at least 2 sites positive for AdV and 20 patients had ≥ 3 sites positive for AdV which is consistent with the definition of dADV. In agreement with previous reports the most common subgroup was C followed by A, B and D (14%, 7%, 7% for A, B, D).[6, 11, 12] All patients had severe immune deficiency related to several factors at the time of the first adenoDNAemia: 26 (87%) patients had active GVHD, all patients were on immunosuppressive treatment and 20 (67%) patients were receiving ≥ 0.5 mg/kg/day prednisolone equivalent, 18 patients had lymphocytopenia (lymphocyte count < 0.2×10^9/L) and 8 had neutropenia (neutrophil count < 0.5×10^9/L). Concomitant viral, invasive fungal and bacterial infections were very frequent and diagnosed in 19 (63%), 15 (50%) and 8 (27%) patients respectively (Table 2). This rate was higher than reported in patients with AdV disease but is consistent with the immune deficiency characterizing patients with dAdV.[6, 13]

The two-year cumulative incidence of dAdV was 11% (95%CI: 7-14) in patients who received an unrelated graft. Age of recipients was a risk factor for dAdV in the Cox model (children, HR: 1.81, 95%CI: 1.23-2.68, p=0.0027, p<0.001). Interestingly, our study showed that previous acute GVHD was a risk factor for dAdV both in adults and children who received an unrelated HSCT (Table 3). In children, the second independent risk factor was a diagnosis of Fanconi anemia. Indeed, in our center, many children with this diagnosis underwent HSCT because it is the only curative treatment.[14] An immune deficiency inherent to the disease and an increased susceptibility to tissue damage could both contribute to the increased risk of AdV disease.[14] Adults who received an unrelated cord-blood (UCB) source were also at higher risk of dAdV. CB graft does not carry the lymphoid mature cells required for anti-infectious defence. Therefore, the risk of infection may be increased, in particular in adults who have a slower immune reconstitution.[15] This is the first time UCB has been shown to be a risk factor for dAdV in adults while a large study has recently reported a higher rate of severe infections in 48 UCB of 192 unrelated recipients.[16] Another team recently also described an increased risk of Epstein-barr-virus lymphoproliferative disease among UCB recipients.[17] It is interesting that this higher risk was only seen in patients who received antithymoglobulin (ATG) during the conditioning regimen. Until now, our center, all patients undergoing UCB transplantation received ATG in conditioning regimen. This may explain the increased risk of dAdV in these patients.

Table 1. Transplantation characteristics for unrelated stem cell recipients.

	Patients without adenovirus	*Patients with adenovirus*
Number	247	26*
Median age in years (range)	25 (4-62)	15 (4-50)
Age < 15 years	71	15
Primary disease		
Acute leukemia / Myelodysplasia	156	12
Aplastic anemia	47	10
Fanconi anemia	20	9
Lymphoma / CLL / Myeloma	28	1
Chronic myeloid leukemia	26	3
Other	2	-
Source of stem cells		
Bone marrow	123	11
Peripheral blood stem cells	72	2
Cord blood	54	13
HLA mismatch stem cells	111	15
Second allogeneic graft	7	5
Conditioning regimen		
Reduced intensity*	74	14
Irradiation based	130	10
Antithymoglobulin therapy	180	26
No engraftment or rejection	32	2
Acute GVHD		
Grade 0	78	6
Grade I	51	1
Grade II	92	10
Grade III	16	4
Grade IV	12	5

**Reduced intensity conditioning regimen excluding all conditioning with either irradiation ≥ 10 Gy or busulfan at 16mg/kg or cyclophosphamid ≥ 150 mg/kg/day. Two patients with low viral load were excluded from statistical analysis, a 13-year old child with Fanconi anemia and a 48-year old patient with chronic myeloid leukemia. Both received an unrelated cord-blood transplant and had no GVHD at time of first adenoviremia.*

Table 2. Description of co-morbidities at time of disseminated adenovirus infection (dAdV) diagnosis and outcome.

Patient #	GVHD at dAdV diagnosis	Copathogens at dAdV diagnosis	AdV clearance	Outcome	Time from AdV diagnosis to death or last news (days)	Cause of death
# 1	Gr IV	IA	No	Death	52	AdV, co-infection, rejections
#2	Gr III	CMV#3	No	Death	7	Micro-angiopathy, rejection
#3	No	IA & F, P.Aeruginosa b, HSV*	No	Death	30	AdV, co-infections
#4	No		Yes	Alive	1,440	-
#5	No	F	No	Death	44	AdV, co-infections
#6	Grade II	IA, E. Cloacae b, EBV	No	Death	57	AdV, co-infections & GVHD
#7	Grade IV		No	Death	55	ADV & GVHD
#8	Grade IV		Transient	Death	252	AdV, renal failure
#9	Extensive	IA, EBV	No	Death	44	AdV, co-infections
#10	Extensive	IA, P. aeruginosa b	No	Death	33	AdV, co-infections
#11	Grade III	IA	No	Death	26	AdV, co-infections
#12	Grade IV	IA, CMV#2	Transient	Death	86	AdV, co-infections
#13	Grade IV	CMV#1, EBV	No	Death	46	AdV, co-infections, GVHD
#14	Grade II	EBV	No	Death	21	AdV, co-infections
#15	Grade II	F, E. coli b, HSV	No	Death	48	AdV, co-infections, rejection
#16	No	IA	No	Death	125	AdV, co-infections, GVHD
#17	Extensive	CMV#3	Yes	Death	391	Respiratory distress of unknown origin
#18	Grade II	EBV	Yes	Alive	343	-
#19	Grade III	IA, HSV	No	Death	57	AdV, co-infections, GVHD
#20	Extensive	CMV#2, HSV, RSV	No	Death	58	AdV, co-infections
#21	Grade III	CMV#1, EBV, HSV, RSV	No	Death	33	AdV, co-infections
#22	Extensive	IA	No	Death	28	Hematological relapse
#23	Extensive	K. pneumonia b & p, CMV#2, EBV, HSV	No	Death	5	AdV, EBV lympho-proliferative disease
#24	Extensive	M. avium b & p, CMV#3, HSV, rotavirus	No	Death	104	AdV, bacterial co-infections
#25	Grade II	Rhizopus, P.aeruginosa b, CMV#2, RSV, enterovirus	No	Death	30	AdV, co-infections
#26	Grade II	IA, CMV#2, HSV	Yes	Alive	111	-
#27	No	IA, CMV#1, EBV	No	Death	58	AdV, co-infections
#28	Extensive	IA	No	Death	32	AdV, secondary pancytopenia
#29	Extensive	P.aeruginosa p	Yes	Death	399	Bacterial pneumonia
#30	Extensive	CMV#2	Yes	Alive	135	-

ADV: adenovirus, IA: invasive aspergillosis, F: fungemia, HSV: Herpes simplex virus in spite of acyclovir treatment (clinical or microbiologic acyclovir – resistance), progressive: uncontrolled ADV infection, CR means complete response with negativity of ADV blood PCR, the 2 last patients (#29 and 30) are the related recipient. Co-infections were defined as all infections treated at time of dADV diagnosis. Co-infections included documented viral and probable or proven invasive fungal infections[22] and bacteremia (b) or pneumonia (p), excluding coagulase-negative Staphylococcus. Patients #19 to 30 were diagnosed after 2005. Patients #19; 24 and 30 had real time PCR for AdV < 10,000/ml copies.*

Table 3. Multiple Cox model for disseminated adenovirus risk factors in children and in adults among unrelated recipients.

	Hazard ratio	95% confidence interval	p-value
Children			
Fanconi disease	3.51	1.13 - 11.3	0.03
Grade II-IV AGVHD	4.05	1.23 - 13.4	0.022
Adults			
Cord blood stem cell as source	3.35	1.13 - 9.93	0.029
Grade II-IV AGVHD	4.24	1.21 - 14.89	0.024

The following covariates were tested by Cox analysis: patient age, primary hematological diagnosis, conditioning regimen (reduced vs. standard), the use of anti-thymoglobulin (ATG) within conditioning regimen, number of allogeneic transplantations (first or second), source of stem cells (bone marrow, peripheral blood, CB stem cell) and acute GVHD (grade 0-I versus II-IV) as a time-dependant covariate. Factors achieving a p-value < 0.20 in univariate analysis were included in multivariate analysis. Risk factors for disseminated AdV infection were analyzed separately in adults and children because primary disease and source of stem cells were not similar in these categories: Fanconi disease in 24 out of 86 children and in 5 out of 189 adults; CB in 41 out of 86 children and in 26 out of 189 adults.

All patients who had sufficient estimated life expectancy (n=25) received cidofovir (*Table 4, online supplement*). Cumulative incidence of AdV clearance was 23% (95%CI: 8-38), only after several weeks of cidofovir treatment. As expected, in patients with dAdV, the rate of remission was lower than previously reported in patients with adenoviral disease or infection.[18,19] The apparent slow response to cidofovir raises questions about the real efficacy of cidofovir. But 2 patients who had treatment discontinuation because of uncontrolled GVHD had rapid progression of viral load followed by death. Others have reported spontaneous remission in patients with AdV infection but, to our knowledge, never in cases of disseminated infections. Predictors for AdV clearance were late dADV (> 3 months), related graft and absence of coinfections (Table 4). None of the patients who reached 100,000 copies/ml had a response. In addition, low corticosteroid dosage (< 0.5 mg/kg/d, HR:0.16, 95% CI: 0.03-0.811, p=0.027) and younger age (continuous covariate, HR: 0.87, 95% CI: 0.79-0.96, p=0.008) were also associated with a better response in patients who received an unrelated graft.

One-year survival was dramatically low in unrelated recipients compared with related recipients: 18% (95%CI: 8-41) vs. 100% which is lower than previously reported.[6] Survival was not influenced by clinical signs (pneumonia 27%, 95% CI: 11-62, vs. 18%, 95% CI: 6-57) or number of affected sites (> 2 sites 27%, 95% CI: 7-52 vs. 19%, 95% CI: 7-52). Only patients who responded to cidofovir survived, with a 70% (95% CI: 42-100) survival rate at one year (p<0.0001).

In conclusion, clinical signs of viral infection associated with an adenoDNAemia are always seen in patients who

are severely immunocompromised. In the unrelated recipients who received high dose corticosteroids, AdV should be screened even after the third month post-transplant and a pre-emptive treatment should probably be started in cases of AdV infection. Also, sice cidofovir seems to cure only a minority of patients, probably because of lack of immune reconstitution, some new immunotherapeutic strategies are needed which may improve this poor outcome.[20,21]

Author's Contributions

GS and MR designed the study and were responsible for analysis; GS, SMJ, CS and MR wrote the paper; CS and SMJ performed the virological tests; SMJ and MR collected the data; MR performed statistical analysis; JMM, AB, MR, GS, EG, PR, VR, CF, RDT and AD were responsible for patient care; MR and SMJ contributed equally to this work.

Conflict of Interest

The authors reported no potential conflicts of interest.

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