Chapter 9

Influence of Antithymocyteglobulin dose on outcome in Cytomegalovirus-seropositive recipients of partially T cell depleted stem cell grafts from matched unrelated donors

Ellen Meijer,
Adriaan W. Dekker,
Leo F. Verdonck

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Abstract

Recently, several reports stressed the adverse impact of a positive recipient Cytomegalovirus (CMV) serostatus on outcome in recipients of matched unrelated donor (MUD) grafts. In this study we evaluated whether CMV-seropositive MUD recipients transplanted after 1999, still showed inferior outcome compared to CMV-seronegative recipients. In that year two important changes in transplantation procedure were introduced: 1) reduction of Antithymocyteglobulin (ATG) dose (ATG was given prior to the myeloablative conditioning regimen to prevent non-engraftment), 2) introduction of sequence based typing of HLA-DRB1. In total 80 patients received partial T cell depleted grafts, 36 before 1999 and 44 after 1999. CMV-seropositive patients transplanted before 1999 showed a highly significant inferior outcome compared to seronegative recipients. In contrast, in patients transplanted after 1999 no difference in outcome was observed between the two groups.

Introduction

Cytomegalovirus (CMV) infections in recipients of allogeneic stem cell transplants (SCT) historically have been an important cause of morbidity and mortality, primarily due to CMV pneumonia. This very serious complication occurred mainly in CMV-seropositive recipients, with acute graft-versus-host disease (aGVHD) being an important risk factor[1]. Since the introduction of pre-emptive treatment of CMV reactivations, a positive CMV serostatus no longer is an adverse risk factor for outcome in recipients of matched related donor (MRD) grafts[2-5]. However, in recipients of grafts from matched unrelated donors (MUD), recipient CMV-seropositivity has a major negative impact on survival after SCT[5-8]. Most patients in these studies were treated before 1999. In our transplantation centre two important changes in transplantation procedure were introduced in that year. All patients received partial T cell depleted (TCD) grafts and were pre-treated with Antithymocyteglobulin (ATG) prior to the myeloablative conditioning regimen to prevent non-engraftment. In April 1999 ATG dose was lowered from 20 mg/kg to 8 mg/kg. Furthermore, in January 1999 sequence based typing (SBT) of HLA-DRB1 was introduced. Both changes may result in an improved immune reconstitution post-transplant. Low-dose ATG by a direct effect on T lymphocyte counts and high resolution HLA-DRB1 typing by a decreased incidence of GVHD. Considering the immunosuppressive effect of (latent) CMV infection[9-10], this may have a positive impact on outcome in CMV-seropositive SCT recipients, which is analysed in the present study.

Patients and methods

Patients For this study data of 80 consecutively treated patients receiving stem cells from MUDs were analysed. Thirty-six patients were treated from July 1990 until January 1999 and 44 from January 1999 until January 2002. Patients with acute leukaemia's (AL) in first complete remissions (CR), chronic myeloid leukaemia (CML) in first chronic phase (CP) and untreated severe aplastic anaemia (SAA) were considered low-risk. All patients with AL and CML in more advanced stages and other diseases were considered high-risk. TRM was defined as any mortality after transplantation, except relapse. Transplantations were performed at the Department of Haematology of the University Medical Centre Utrecht.

Patients were treated according to clinical protocols approved by the local investigation review board after informed consent was obtained.

Transplantation procedure The conditioning regimen consisted of cyclophosphamide (60 mg/kg/day for 2 days), followed by total body irradiation (600 cGy/day for 2 days) with partial shielding of the lungs (total lung dose 850 cGy). The graft was infused after the second TBI fraction (day 0). Antithymocyteglobulin (Thymoglobulin™, Sangstat, Amstelveen, the Netherlands) was given to MUD patients before cyclophosphamide was infused, in a dose of 4 mg/kg/day intravenously for 5 days. Due to a change in national treatment protocols, ATG dose was lowered to 2 mg/kg/day for 4 days from April 1999. Post-transplant immunosuppression consisted of cyclosporin which was discontinued within 3 months after transplantation, when no active GVHD was present. Infection prevention for all patients consisted of ciprofloxacin, fluconazole and amphotericin B given orally until granulocyte counts exceeded 500 cells/μl. Cephalothin was given intravenously for 10 days from day +3. Furthermore co-trimoxazole and (val)acyclovir were given orally from day +1 until 12 months post-BMT or longer in case of active GVHD, in a dose of 480 mg b.i.d. and 500 mg b.i.d., respectively.

CMV monitoring Until April 2001 CMV monitoring was pp65 based as described[5]. Since then monitoring was performed using a real-time Taqman™ CMV DNA PCR. CMV reactivation was defined as CMV pp65 antigenemia of $\geq$ 1 positive staining granulocyte/150.000 cells or CMV DNA viral load (VL) of > 1000 copies/ml.

CMV disease Patients with symptoms of pneumonia, gastritis or enteritis underwent bronchoscopy, gastroscopy or sigmoidoscopy, respectively. CMV pneumonia/gastritis/enteritis was defined histologically by typical cytopathic effects and immunohistochemically by immunofluorescence with use of monoclonal antibodies to immediate early CMV antigens in biopsy specimens. When cultures of BAL fluid, saliva, urine and buffy coat were performed in case of infectious complications, these included always CMV cultures, irrespective of CMV serostatus.

Ganciclovir therapy CMV-seropositive patients who demonstrated CMV reactivation or who were treated with high-dose corticosteroids for aGVHD grade II-IV received pre-emptive or prophylactic therapy, respectively, with ganciclovir in a dose of 2.5 mg/kg intravenously twice a day for 14 days. When patients were symptomatic (unexplained fever or symptoms compatible with CMV disease), CMV antigenemia/VL was rising or remained positive after 14 days of treatment, ganciclovir dose was doubled or foscarnet treatment was

started instead of ganciclovir in a dose of 60 mg/kg twice a day for 14 days. CMV disease was treated with ganciclovir 5 mg/kg twice a day for at least 14 days and continued until symptoms resolved and antigenemia/VL became negative. In case of disease progression or rising antigenemia/VL foscarnet treatment was started instead of ganciclovir in a dose of 60 mg/kg twice a day. Furthermore, treatment with CMV specific immunoglobulins was added to antiviral therapy in patients with CMV pneumonia.

HLA-matching HLA-A and B matching was based on serological typing and HLA-C, DRB1 and DQB1 matching was based on low resolution molecular typing with sequence specific primers. Since January 1999 and July 2000 SBT of HLA-DRB1 and HLA-A, B and DRB1, respectively, was performed as well.

BMT In vitro partial TCD of the marrow was performed using the Soy Bean Agglutinin/Sheep Red Blood Cell technique until 1997, thereafter, the immunorosette depletion technique was used as described[5]. After this maximal T cell depletion procedure the residual number of T cells was counted and nonmanipulated T cells (from a small BM fraction that was set apart) were added to obtain the desired fixed low number of T cells (1-5 x 10^5 T cells/kg recipient weight).

Statistical analysis Overall survival (OS) was estimated by the Kaplan-Meier method. Probability of transplant related mortality (TRM) was calculated by the cumulative incidence procedure, death from relapse being the competing risk. Univariate analyses were performed using the log rank test. Variables which showed to influence OS/TRM at a level of $p<0.1$ were used in a multivariate Cox regression analysis. P values from regression models were calculated with the Wald test. Pre- and post-transplant variables analysed were: CMV serostatus of recipient (positive vs negative), CMV serostatus of donor (positive vs negative), patient age (continuous), risk status (high vs low), patient/donor sex (m/f vs other), T cell count of the graft ($< 2 \times 10^5$/kg vs $\geq 2 \times 10^5$/kg), ATG dose (high-dose vs low-dose), aGVHD (II-IV vs other), chronic GVHD (extensive vs other), CMV reactivation (yes vs no).The post-transplant variables 'aGVHD' and 'cGVHD', 'CMV reactivation' were as well analysed as time-dependent covariates. Calculations were performed using SPSS/PC+ 10.0 (SPSS Inc, Chicago Il, USA).

Results

Patient characteristics (Table 1). Thirty-six patients were treated before 1999 (historical group) and 44 since 1999 (recent group). In both groups one patient was excluded from analysis because of non-engraftment. Acute GVHD grade II-IV was more often diagnosed in the historical group compared to the recent group (40% vs 23%, respectively, ns) as was extensive cGVHD (17% vs 7%, respectively, ns). Most important is the percentage of CMV reactivations which was only 14% in the recent group compared to 31% in the historical group (p=0.063). Furthermore, most patients in the historical group experienced recurrent CMV reactivations (6 of 11 patients), compared to 1 of 6 patients in the recent group. Two patients in the historical group showed ganciclovir related neutropenia, compared to none in the recent group. Median follow up of survivors was 68 months (range: 45-115) in the historical group and 20 months (range: 6-43) in the recent group.

OS and TRM Figure 1 shows OS and TRM in patients transplanted before and after 1999 according to recipient CMV serostatus. After univariate analyses, the only negative risk factor for OS in the historical group was a positive recipient CMV serostatus (p=0.009), while recipient CMV-seropositivity, CMV reactivation and aGVHD grade II-IV were adverse risk factors for TRM in the historical group. However, after multivariate analysis, only recipient CMV-seropositivity adversely affected TRM (p=0.004). In the historical group 11/17 CMV-seropositive patients died from fatal viral (CMV pneumonia: n=3) and fungal infections, compared to 1/18 (CMV disease: n=0) in the recent group (p=0.005). In the historical group, survival analyses were also performed for all 4 recipient/donor CMV serostatus combinations separately (R+/D+, R+/D-, R-/D+, R-/D-), showing no effect of donor serostatus in the CMV-seropositive recipients. In the group treated since 1999 recipient CMV-seropositivity did not affect outcome anymore.

When analyses were performed among CMV-seropositive patients, only ATG dose was a negative risk factor for outcome (OS: p=0.06; TRM: p=0.007).

Discussion

This study shows that lowering ATG dose and introducing SBT of HLA- DRB1 (and later also HLA-A and B) had an important impact on OS and TRM in CMV-seropositive MUD recipients of partial TCD grafts. HLA-matching based on high resolution DNA typing resulted in a decreased incidence of acute and chronic GVHD (although ns). Since ATG dose was the only risk factor for outcome in seropositive patients and GVHD showed to have no impact, we postulate that lowering the ATG dose has been the most important factor diminishing mortality in CMV-seropositive recipients. Until April 2001 CMV monitoring was performed using

Figure 1

Probability of OS and TRM in MUD recipients treated before and after 1999 according to CMV serostatus.

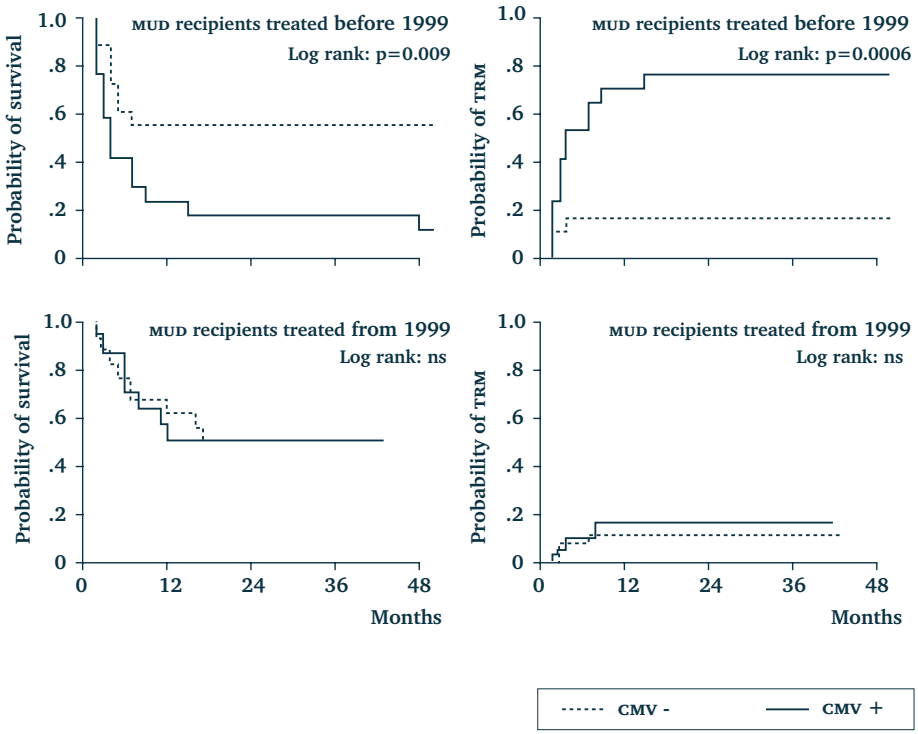

Table 1 Patient characteristics

	Before 1999	After 1999	P
No. of patients	35	43	
Age, yr (range)	31 (17-47)	33 (17-55)	ns
Diagnosis (%)			ns
AML	6 (17)	10 (23)	
ALL	8 (23)	12 (28)	
CML	10 (29)	13 (30)	
SAA	7 (20)	1 (2)	
Other	4 (11)	7 (16)	
CMV serostatus R/D (%)			ns
+/+	6 (17)	9 (21)	
+/-	11 (31)	9 (21)	
-/+	7 (20)	8 (19)	
-/-	11 (31)	17 (40)	
Risk status (%)			ns
Low	13 (37)	9 (21)	
High	22 (63)	34 (79)	
aGVHD (%)			ns
No-I	21 (60)	33 (77)	
II-IV	14 (40)	10 (23)	
cGVHD (%)			ns
No-L	29 (83)	40 (93)	
E	6 (17)	3 (7)	
CMV reactivation (%)			0.06
Yes	11 (31)	6 (14)	
No	24 (69)	37 (86)	
Recurrent CMV reactivations			ns
One	5	5	
Two or more	6	1	
Time to first reactivation			ns
median days (range)	31 (20-52)	50 (11-78)	
Granulocytes >500 x 10^6/L			ns
recovery	100%	100%	
median days (range)	23 (13-48)	19 (12-92)	
Platelets >50 x 10^9/L			ns
recovery	88%	95%	
median days (range)	33 (17-148)	28 (12-208)	

R/D = recipient/donor; L = limited; E = extensive.

the CMV pp65 antigenemia assay, thereafter monitoring was based on a real-time TaqMan™ CMV DNA PCR. This method is known to be more sensitive[11-13] compared to the antigenemia assay, however, the incidence of CMV reactivations was still lower in the recent group compared to the historical group. In conclusion, CMV-seropositive MUD recipients of partial TCD grafts treated since 1999 show survival rates comparable to CMV-seronegative recipients. This effect largely resulted from a decrease in dosage of ATG, although high resolution HLA-antigen typing may have contributed. Low-dose ATG did not result in an increase in non-engraftment, while high-dose ATG is associated with an increase in acute toxicity. Therefore, we recommend when ATG is used in the setting of TCD MUD transplantation, to use the low-dose of 8 mg/kg.

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