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High-dose acyclovir and pre-emptive ganciclovir in prevention of cytomegalovirus disease in pediatric patients following peripheral blood stem cell transplantation

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Summary:

Cytomegalovirus (CMV) disease remains an important cause of morbidity and mortality in patients undergoing hematopoietic stem cell transplantation (HSCT). We evaluated high-dose acyclovir (HDACV) and pre-emptive ganciclovir to prevent CMV disease in 76 children who underwent peripheral blood stem cell transplantation (PBSCT) and were at risk for CMV reactivation and disease (both recipient and donor seropositive) from May 1998 to April 2003. All received HDACV from day −9 to 6 months post transplant in conjunction with weekly CMV pp65 antigenemia monitoring. The incidence of antigenemia in this cohort was 19.7%, at a median of 22 days post-PBSCT. The frequencies were 26.4 and 4.4% in allogeneic and autologous groups, respectively ($P = 0.03$). Patients with nonmalignant disease had higher CMV antigenemia than those with malignant disease (30.8 *vs* 8.1%, $P = 0.02$). Age at PBSCT, sex, graft-versus-host disease (GVHD) prophylaxis regimen and presence of acute GVHD did not affect the risk of CMV antigenemia. None of the patients who had positive pp65 antigenemia developed CMV disease during the study period. We conclude that pp65 antigenemia-guided HDACV and pre-emptive ganciclovir may prevent CMV disease in children undergoing PBSCT.
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Cytomegalovirus (CMV) disease remains an important cause of morbidity and mortality in patients undergoing hematopoietic stem cell transplantation (HSCT), particularly in allogeneic recipients.[1,2] Increased risk for CMV disease following HSCT is associated with recipient CMV serology pre-transplant, T-cell depletion, type of transplant, acute graft-versus-host disease (aGVHD) and HSCT from donors other than HLA-identical siblings.[1,3–6] Mortality attributable to CMV reactivation in HSCT patients could be as high as 50%.[7] The availability of effective antiviral therapy such as ganciclovir (GCV) has led to dramatic improvements in the outcome of CMV infection in patients undergoing transplantation.[8] Two different strategies have been developed for prevention of CMV disease in this population. These are either prophylaxis of all 'at-risk' patients for a defined period or pre-emptive treatment with ganciclovir of selected 'at-risk' patients based either on laboratory markers indicative of subclinical infection or the presence of specific risk factors.[9] Both acyclovir (ACV) and GCV are effective in the prophylaxis of patients at high risk for CMV disease.[10,11] The adverse effects of GCV, including myelosuppression and nephrotoxicity limit its prophylactic usage for all transplant recipients.[8,12] Thus, it is important to identify the patients who will be started on 'pre-emptive' therapy. Such patients should be identified early and given antiviral therapy to prevent the onset of disease. Regular blood monitoring for CMV antigenemia or DNA are essential for pre-emptive therapy. High-dose ACV (HDACV) (1.5 g/m^2/day) may be an alternative way to avoid the disadvantages of GCV in prophylaxis of CMV disease.[10,13,14]

We evaluated HDACV and pre-emptive GCV to prevent CMV disease in pediatric patients following PBSCT.

Patients and methods

Patients

During a period of almost 5 years (from May 1998 to April 2003), 76 pediatric patients with a follow-up longer than 20 days underwent PBSCT in the Stem Cell Transplantation Units at Akdeniz and Ege Universities. All patients but one received their first transplant. Characteristics of patients analyzed are shown in Table 1. All patients and donors were positive for CMV antibody before transplantation. Most of the patients without malignancy (70.0%) had a hemoglobinopathy.

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Table 1 Patient Characteristics[a]

Number of patients	76
Median age in years (range)	7.5 (range,1–18)
Sex	
Male	43 (56.6)
Female	33 (43.4)
Underlying disease	
Acute leukemia[b]	18 (23.7)
Lymphoma[c]	5 (6.6)
Solid tumors[d]	14 (18.4)
Nonmalignant disease[e]	39 (51.3)
Type of transplant	
Autologous	23 (29.2)
Allogeneic	53 (70.8)
Conditioning regimen	
None[f]	3 (3.9)
CT	49 (64.5)
CT + RT	1 (1.3)
CT + ATG[g]	19 (25)
CT + RT + ATG	4 (5.3)
aGVHD grade[h]	
None	35 (66)
Grade I	9 (17)
Grade II	3 (5.7)
Grade III	1 (1.9)
Grade IV	5 (9.4)
cGVHD[h]	
No	47 (88.7)
Yes	6 (11.3)

[a]Data are *n* (%). GVHD, CT, RT and ATG indicate graft-*versus*-host disease (a-acute, c-chronic), chemotherapy, radiotherapy and anti-thymocyte globulin, respectively.
[b]Acute leukemia includes 11 lymphoblastic and seven myeloblastic leukemias.
[c]Lymphoma includes two non-Hodgkin and three Hodgkin lymphomas.
[d]Solid tumors include six neuroblastomas, four rabdomyosarcomas and four germ cell tumors.
[e]Nonmalignant diseases include 25 hemoglobinopathies, five immunodeficiencies, four fanconi aplastic anemias, four aplastic anemias, one adrenoleukodystrophy, amegakaryocytic thrombocytopenia, hemophacytosis and myelodysplastic syndrome.
[f]Patients with severe combined immune deficiency were not given any conditioning regimens.
[g]ATG, rabbit ATG, totally 30 mg/kg.
[h]Allogeneic recipients only ($n = 53$).

HSCT

Three patients with severe combined immune deficiency were not given any conditioning regimens. Four different preparative regimens were used for the others. As multidrug chemotherapy ($n = 49$), cyclophosphamide and busulphan-based protocols were used for allogeneic transplantation ($n = 27$), while melphelan and busulphan-based combinations were given for autologous transplantation ($n = 22$). One patient with a diagnosis of Hodgkin's lymphoma underwent total nodal irradiation and melphelan and cyclophosphamide as the conditioning regimen. A total of 21 patients received anti-thymocyte globulin (ATG) with ($n = 4$) or without ($n = 19$) thoracoabdominal irradiation as prophylaxis against graft rejection and GVHD. Cyclosporin A combined with short-term methotrexate was used for prevention of GVHD. Except for six patients, three of who had one HLA antigen-mismatched related donor transplant and three of who had haploidentical transplantation, all donors were fully-matched siblings or parents. Ciprofloxacin, flucanazole and sulfamethoxasole/trimetoprim were given for prophylaxis against bacterial, fungal and *Pneumocystis carinii* infections.

CMV prophylaxis

All patients received ACV 10 mg/kg i.v. three times a day or, when feasible 20 mg/kg p.o. four times a day from day −9 to day 30 (or until achieving engraftment). Oral ACV prophylaxis was continued until 6 months post transplant. If the patient had aGVHD, it was continued until resolution of GVHD. All patients also received intravenous immunoglobulin (IVIG) 500 mg/kg once a week from day −1 to day 180.

Definitions and CMV surveillance/pre-emptive therapy for CMV disease

CMV infection was defined by a positive pp65 antigenemia assay or CMV DNA detection in two consecutive blood samples. CMV disease was defined as an otherwise unexplained organ dysfunction with CMV infection. CMV antigenemia (pp65) assay was investigated in peripheral blood leukocytes (PBLs) according to the manufacturer's recommendations (CINAkit, Argene-Biosoft, France) at least once a week, starting on day −9. Routine antigenemia assays were continued until day 180 post transplant, irrespective of a positive test. Results were expressed as the number of CMV pp65-positive cells per 2×10^5 PBLs and the test was considered positive when at least one fluorescent cell was observed. If the white blood cell count was not sufficiently high for the pp65 assay, CMV PCR was used. A result with 10 or more, or less than 10, positive cells per 200 000 was defined as high-risk or low-risk, respectively. Patients in the high-risk group were treated with an induction dose of GCV 5 mg/kg i.v. twice a day for 14 days and then followed by 5 mg/kg i.v everyday until negative CMV antigenemia. Patients with organ involvement were treated additionally with intravenous immunoglobulin 2 g/kg. GCV was not started for patients in the low-risk group who had only one positive assay. Administration of GCV in the low-risk group with increasing positive cells but not more than 10 without signs of organ involvement in consecutive determinations was optional.

Statistical analysis

The frequency of CMV antigenemia was measured at 100 days post transplantation. SPSS 10.0 Software was used for statistical analysis. A backward stepwise logistic regression test was used to determine factors that independently affected the probability of positive antigenemia. Comparisons of nominal data and numeric data were performed by χ^2 tests and Mann–Whitney *U* test, respectively. Survival estimates were calculated by Kaplan-Meier methods, and 95% confidence intervals (CI) were determined from the

standard errors. Differences between groups were tested by log rank test of significance.

Results

Patients and transplant outcomes

Transplant outcomes are shown in Table 2. Actuarial survival rate with a median follow-up of 14 months (1–59) is 57.5±6%. Of 76 transplant patients, 48 patients are alive with a median follow-up 20 months (2–53). One patient died of regimen-related toxicity (pulmonary hemorrhage) before engraftment. Two patients did not have initial engraftment and died of bacterial infection. One patient with beta thalassemia major had successful initial engraftment, but developed graft failure 6 months after transplant and died of bacterial infection. A total of 15 patients died of relapse or progressive disease. The three remaining patients died of infections (bacterial two, fungal one). One patient died of intracranial hemorrhage secondary to trauma before platelet engraftment. One patient with a diagnosis of thalassemia major developed hepatic insufficiency due to iron overload and died 22 months after transplant. Nine patients developed grade II–IV aGVHD and received methylprednisolone. Three died despite methylprednisolone and ATG for progressive GVHD. None of the patients without aGVHD were given steroids.

Probability and risk factors of CMV antigenemia

Of 76 patients in this cohort, 15 (19.7%) developed CMV antigenemia at a median onset of 22 days post-PBSCT (range, 5–60 days). The median peak level was seven cells per 200 000 (range, 1–46). One patient developed a second positive antigenemia. The remaining 14 patients had only one positive test. Of these, seven had high-risk antigenemia. Since antigenemia resolved in all patients started on preemptive therapy at the end of 2-week GCV regimen, none received GCV after induction. None of the patients developed rising antigenemia during GCV therapy. We did not observe any CMV disease during the study period, either before 100 days or after 100 days. There was no recurrence of antigenemia after GCV treatment. Potential risk factors evaluated by this study are shown in Table 3. Age at transplantation, sex, GVHD prophylaxis using ATG, aGVHD grade II–IV and cGVHD were not significant risk factors for CMV antigenemia. The probability of positive antigenemia in the allogeneic group was significantly higher than that in the autologous group (93.3 *vs* 7.1%, $P=0.03$). Underlying disease was also a potential risk factor for CMV reactivation by univariate analysis. Multiple regression analysis, however, revealed neither transplant type nor underlying disease to be an independent risk factor for CMV reactivation. Overall survival (OAS) for patients with positive antigenemia was not significantly different compared with OAS for the antigenemia negative group (65±12 *vs* 55%±7, $P=0.6$). None of the patients who died after transplantation had CMV antigenemia at the time of death. No patients developed impaired renal function attributable to HDACV. GCV was not stopped in any patients because of neutropenia as an adverse effect.

Table 2 Transplant outcomes

	CMV pp65 Ag (+)	*CMV pp65 Ag (−)*	*P*
Median follow-up (months)	20 (2–40)	10 (1–53)	
Overall survival	65.4±12%	55.5±7%	0.6
Acute GVHD grade II–IV	28.6%	12.8%	0.2
Chronic GVHD	14.3%	10.2%	0.6
Causes of death			
Relapse or progression	3	12	
Infections			
Bacterial	—	4	
Fungal	—	1	
aGVHD	1	2	
Idıopatic IP/ARDS[a]	—	1	
Others	1	3	

[a]IP/ARDS indicates interstitial pneumonitis/acute respiratory distress syndrome.

Table 3 Risk factors for CMV antigenemia[a]

Risk factor	*Number of patients*		*Rate of antigenemia (%)*	*P*
	At risk	*Antigenemia post-PBSCT*		
Cohort	76	15	19.7	
Age at transplantation (years)	31	5	16.1	0.5
<5	45	10	22.2	
⩾5				
Sex				0.2
Male	43	6	13.9	
Female	33	9	27.3	
Underlying disease				0.02
Malign	37	3	8.1	
Nonmalign	39	12	30.8	
Type of transplant				0.03
Allogeneic	53	14	26.4	
Autologous	23	1	4.4	
ATG[b]				0.7
No	30	7	23.3	
Yes	23	7	30.4	
Grade II–IV aGVHD[b]				0.2
No	44	10	22.7	
Yes	9	4	44.4	
cGVHD[b]				0.6
No	47	12	25.5	
Yes	6	2	33.3	

[a]GVHD and ATG indicate graft-versus-host disease (a-acute, c-chronic) and anti-thymocyte globulin, respectively.
[b]AlloPBSCT recipients only ($n=53$).

Discussion

Our results using HDACV prophylaxis and antigenemia-guided pre-emptive GCV treatment are encouraging. Under HDACV prophylaxis, the frequency of CMV reactivation was 19.7%. No CMV disease was observed in the study period. Although CMV is less sensitive to inhibition by ACV than the other herpes viruses such as herpes simplex or varicella-zoster viruses *in vitro*, improved survival with HDACV has been shown in a small number of adult studies.[13–16] One study showed, however, no clear benefit with HDACV followed by prophylactic GCV.[17] There is no randomized study comparing prophylactic HDACV in combination with pre-emptive GCV. Nakamura *et al*[16] evaluated HDACV and pre-emptive GCV to prevent CMV disease in myeloablative and nonmyeloablative allogeneic SCT in a nonrandomized study. They used HDACV for 3 months post transplant in conjunction with weekly CMV pp65 antigenemia monitoring and preventive treatment with IVIG (non-CMV specific) and GCV. In that protocol, the actuarial probability of developing CMV disease was 6.3%. There was one death clearly attributable to CMV. We did not use visceral tissue samples for detecting CMV disease. Although we may have not detected some mild cases of CMV disease, there were no deaths attributable to CMV disease according to routine post-mortem tissue investigations. Other explanations for the lack of CMV disease in this study may be the stem cell source used for transplantation and the length of prophylactic HDACV use. It is well documented that improved immune reconstitution occurs after allogeneic PBSCT compared with allogeneic bone marrow transplantation (alloBMT) both *in vitro* and *in vivo*.[18–20] We used HDACV for 6 months post transplant. We believe that the use of HDACV for 6 months after transplantation is effective for protection of CMV reactivation and disease.

There is no specifically designed, randomized prospective study that compares HDACV to GCV for prophylaxis in patients at risk of CMV antigenemia. Although the number of patients in both groups is relatively small, the Minnesota group reported no apparent difference between these two drugs.[4] Of seropositive alloBMT recipients, 63% developed antigenemia in our study, compared with a reported 41 and 79% antigenemia in similar patients randomized to receive GCV prophylaxis and placebo, respectively.[21]

Defined levels of antigenemia as a trigger for pre-emptive therapy vary.[4,6,22–25] GCV was started at any antigenemia with or without some stratification criteria, such as high-dose steroids, unrelated donor, in many protocols.[6,16,22,23,25] Other issues that are not clear are the dose and timing of GCV administration to achieve an optimal pre-emptive approach. Koehler *et al*[22] used GCV 10 mg/kg/day starting with any level of antigenemia and continued until antigenemia resolved in pediatric patients. In adult practice, pre-emptive treatment usually starts with an induction period consisting of 5–10 mg/kg/day and followed by a maintenance period until negative antigenemia is documented. The induction period is between 1 and 2 weeks.[6,23,25–27] Kanda *et al*[6] used antigenemia-guided pre-emptive therapy with initial low-dose GCV in a cohort with a risk stratification. The incidence of CMV disease in that study was 1.4% for both early and late disease. They suggested response-oriented and antigenemia-guided pre-emptive therapy with initial low-dose GCV. The induction period may be extended up to 3 weeks or longer for poor response patients.[6] Nonetheless, it should be noted that the total amount and duration of high-dose GCV in the induction period may affect the incidence of adverse effects. Although maintenance treatment was given until antigenemia resolves, dose modifications in this period could be made according to changes in the antigenemia value in many protocols. Boeckh *et al*[23] continued GCV until day 100 after transplant with a dose of 5 mg/kg/day, 6 days a week. In that study, the incidence of CMV disease before and after day 100 was 3.8 and 13.1%, respectively. In that study, fungal infection incidence was 5.0%. We used high-dose GCV (10 mg/kg/day) during a 2-week induction period in our protocol. Although we planned maintenance therapy with the lower dose, this was not used, because all patients with CMV reactivation responded well at the end of induction period and relapse was not observed. There is one death attributable to documented invasive fungal infection (1.3%).

We conclude that HDACV and pre-emptive high-dose GCV for 2 weeks is associated with effective and safe control of CMV reactivation in pediatric patients undergoing PBSCT. Now, we are planning a risk-adapted approach, based not only on the antigenemia value but also on the presence of aGVHD, steroid use and donors other than fully match siblings, with HDACV for prophylaxis and a 7-day induction of pre-emptive GCV to prevent CMV reactivation and disease.

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