Rising antigenemia levels may be misleading in pre-emptive therapy of human cytomegalovirus infection in allogeneic hematopoietic stem cell transplant recipients

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Background and Objectives. Hematopoietic stem cell transplant (HSCT) recipients often show rising levels of antigenemia during pre-emptive ganciclovir treatment of human cytomegalovirus (HCMV) infection. This raises some doubts about the therapeutic decisions to be taken.

Design and Methods. Three groups of HSCT recipients with HCMV infection undergoing anti-viral treatment were identified: group A, showing increasing antigenemia and decreasing viremia and DNAemia; group B, with simultaneous increases in antigenemia, viremia, and DNAemia; and group C, with decreasing levels of all 3 viral markers. Viral load, determined as levels of antigenemia, viremia and DNAemia, was monitored for 3 months post-transplantation in all groups.

Results. Group A HSCT recipients showed antigenemia peaks 2-11 days after the onset of treatment, reaching negative levels only 25-30 days thereafter, whereas viremia and DNAemia started to drop earlier. Group B patients, mainly including HSCT recipients with grade II-IV acute GvHD treated with steroids prior to and during antiviral treatment, showed increasing levels of all three viral parameters until 5-10 days after the start of treatment; the levels dropped to negative values 25-30 days thereafter. Group C patients, who acted as controls, progressively cleared virus from blood as an early result of antiviral therapy.

Interpretation and Conclusions. Antigenemia is not the best assay to guide pre-emptive therapy. Group A patients, who have an isolated increase of antigenemia, do not require a change of the ongoing antiviral therapy. Whether better control of infection could be obtained in group B patients by either reducing immunosuppressive therapy (when possible) or adopting combination therapy remains to be determined.

Key words: human cytomegalovirus, pre-emptive therapy, stem cell transplantation, antigenemia.

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A paradoxical phenomenon consisting of increasing antigenemia during antiviral treatment of human cytomegalovirus (HCMV) infection has been reported for several years in both solid organ transplant (SOT)[1,2] and allogeneic hematopoietic stem cell transplant (HSCT)[3,4] recipients. While rising antigenemia (in the absence of antiviral drug resistance) in SOT recipients with primary HCMV infection has been consistently reported to be associated with decreasing viremia and DNAemia,[1,2] in HSCT recipients rising antigenemia has been reported, in different clinical situations, to be sometimes associated with simultaneous rises in viremia and DNAemia.[4] This finding represents a true increase in viral load rather than a dissociated increase in antigenemia levels only. The pathogenetic basis of these two different clinical conditions has been recently elucidated through *in vitro* studies which demonstrated that an isolated increase of antigenemia is due to an excess synthesis of HCMV pp65 in a few infected endothelial cells, partially escaping the viral DNA replication block induced by ganciclovir treatment, with transfer of pp65 to adhering leukocytes. In the contrast, the simultaneous increase of all viral parameters (viral load) is an expression of complete HCMV replication, occurring in endothelial cells despite the use of antiviral treatment. This may be due either to the presence of a ganciclovir-resistant HCMV strain or to intensive immunosuppressive treatment (mainly utilizing steroids), enhancing viral replication thus increasing levels of circulating virus and viral products, associated with transfer of pp65 to leukocytes adhering to endothelial cells.[5] In the present report, we analyzed the markedly different patterns of parameters used to monitor HCMV infection in HSCT recipients. This analysis allowed us to identify 3 different groups of patients. In the *typical* group (group C, reference group), including the majority of patients, the levels of HCMV viremia, antigenemia and DNAemia all decreased rapidly following the start of treatment with ganciclovir; in the

other 2 *atypical* groups, one (group A) showed an isolated increase of antigenemia, while the other one (group B) showed an increase of all viral parameters (antigenemia, viremia, and DNAemia) following onset of treatment with ganciclovir. We compared the viral parameters in these 3 groups with those of a fourth group (group D) of SOT recipients with primary HCMV infection in whom only rising antigenemia was found during GCV therapy.

The major aim of this study was to identify variables associated with the atypical responses to antiviral treatment observed in HSCT recipients of groups A and B. In SOT recipients, primary HCMV infection, occurring in seronegative recipients of solid organs from seropositive donors, has been found to be the clinical condition consistently associated with rising antigenemia during GCV therapy.[1,2] The different responses to antiviral treatment need careful interpretation: group A patients do not require a switch of antiviral therapy from ganciclovir to an alternative antiviral drug, such as foscarnet or cidofovir, or to combination antiviral therapy, both possibly associated with higher toxicity. In contrast, in order to obtain optimal control of viral replication, group B patients might benefit from a change in intervention strategy (such as a reduction of immunosuppression, when possible, or combination antiviral therapy) although this strategy remains to be properly addressed and its efficacy proven.

Design and Methods

Patients

We retrospectively considered a total of 185 HSCT recipients (127 referred to Pediatric Oncohematology and 58 adult patients referred to the Division of Hematology), transplanted between January 2000 and June 2004. HCMV seronegative donor/recipient pairs were excluded from the study. Eighty-three patients were not treated either because they did not have a positive test for HCMV infection or because they had only one. The remaining 102 patients who were given antiviral treatment were considered for the analysis. Of these, 54 (most of them) were treated in the pediatric ward, while 48 were treated in the Division of Hematology for adult patients. The proportion of pediatric patients requiring treatment for HCMV infection was 54/127 (42%), while as many as 48/58 (83%) adult patients received antiviral treatment. HSCT recipients were divided into 3 groups according to the type of response to antiviral therapy. Group A comprised 32 (31.4%) patients with an isolated increase of antigenemia during treatment; group B comprised 10 (9.8%) patients with increases of all markers (antigenemia, viremia, DNAemia) of viral load during treatment; and group C was formed of the 60 (58.8%) patients with prompt decrease in all markers of viral load, as a result of treatment. The pediatric and adult patient populations were homogeneously represented in the composition of the 3 groups, 17 (31.5%) vs 15 (31.2%) patients in group A, 5 (9.2%) vs 5 (10.4%) patients in group B, and 32 (59.3%) vs 28 (58.4%) patients in group C, respectively. The characteristics of the 102 patients enrolled in this study are reported in Table 1. For comparison, a small group (group D) of SOT recipients (n=8), who had received either heart or heart-lung transplantation, with only rising antigenemia during primary HCMV infection treated with ganciclovir, was also considered.

Table 1. Patients' characteristics.

Parameter	No. of patients Pediatric[a] (n=54)	Adults (n=48)
Median age (years, range)	9 (1-23)	43 (19-55)
Sex M/F	33/21	31/17
Diagnosis[b]		
ANLL	11	19
ALL	15	9
CML	1	10
JMML	4	0
MDS	6	7
Myeloma	0	2
NHL	3	0
Sickle cell disease	2	0
Thalassemia	7	0
Others[c]	5	1
HCMV serology[d]		
R+/D-	22	10
R-/D+	3	2
R+/D+	29	36
Conditioning regimen[e]		
Chemotherapy based	36	26
TBI based	15	12
TBI+ATG-based	3	10
Donor type		
HLA-identical sibling	27	34
Unrelated volunteer	27	14
Source of stem cells		
Bone marrow	48	23
Peripheral blood	5	25
Cord blood	1	0
Grade II-IV GVHD	26	13

[a]A few patients 18-24 yrs old were referred to the Pediatric Ward; [b]ANLL: acute non-lymphoblastic leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; JMML: juvenile myelo-monocytic leukemia; MDS: myelodysplastic syndrome; NHL: non-Hodgkin's lymphoma. [c]Others include: 1 Hodgkin's lymphoma, 1 Diamond-Blackfan anemia, 1 Bernard-Soulier syndrome, 1 rabdomyosarcoma, 1 severe aplastic anemia, 1 breast cancer. [d]R, recipient; D, donor. [e]TBI, total body irradiation; ATG, anti-thymocyte globulin.

Assays for viral load quantification

HCMV infection was defined as active HCMV replication in blood in the absence of clinical manifestations

or organ function abnormalities, whereas HCMV disease required documentation of HCMV infection, together with clinical symptoms and/or organ function abnormalities.[6] All patients were monitored weekly (in the absence of HCMV infection) or twice weekly (during HCMV infection) for 3 months after HSCT. Subsequently, monitoring of HCMV infection was performed monthly during routine clinical controls or in the presence of clinical signs or symptoms suggestive of HCMV disease. The donor/recipient serological status was determined by enzyme-linked immunosorbent assays prior to transplantation, using previously reported methods.[7] The HCMV antigenemia was quantified by counting at the fluorescence microscope the number of pp65-positive peripheral blood leukocytes (PBL) on PBL cytospin preparations (each containing 2×10^5 PBL) that were fixed and stained with a pool of pp65-specific monoclonal antibodies, according to a previously reported procedure.[8,9] Viremia, i.e. the number of PBL carrying infectious virus, was quantified by inoculating 2×10^5 PBL onto monolayers of human embryonic lung fibroblasts (HELF), staining 16-24 hours later with an anti-p72 monoclonal antibody and then counting the number of p72-positive HELF nuclei.[10] Finally, HCMV DNA was quantified in whole blood (10 μL) by quantitative polymerase chain reaction (PCR) analysis, as described previously.[11,12]

Antiviral treatment

All HSCT recipients started antiviral treatment for HCMV infection according to the same protocol of preemptive therapy, as reported previously.[13,14] Briefly, all patients received ganciclovir intravenously at a standard dosage of 5 mg/kg of body weight/bid until pp65 antigenemia clearance (i.e., after 2 consecutive blood samples taken 2 to 3 days apart were negative for antigenemia). Ganciclovir therapy was started either immediately, when 2 or more pp65-positive PBLs were detected, or when detection of 1 pp65-positive PBL was confirmed in the following 2 to 3 days at the same or a higher level. In some patients, treatment was initiated after reaching at least 100 HCMV DNA copies/10 μL blood. Ganciclovir resistance was tested by sequencing UL54 and UL97 open reading frames of the HCMV genome, as reported by Baldanti *et al.*[15]

Graft-versus-host disease prophylaxis and treatment

Prophylaxis of graft-versus-host disease (GvHD) consisted of cyclosporine-A (Cs-A) alone for patients receiving the allograft from an HLA-identical sibling, whereas patients who received the transplant from an unrelated donor were given a short course of methotrexate (15 mg/m^2 on day +1, and 10 mg/m^2 on days +3, +6, and +11 after transplantation) and anti-thymocyte globulin (3.75 mg/kg/day from day -4 to day -2) in addition to Cs-A. Patients with acute grade II-IV GvHD were treated with steroids (2-5 mg/kg/day until resolution of clinical symptoms or evidence of progression) as first-line therapy, whereas patients with steroid-resistant disease were mainly treated with extracorporeal photochemotherapy.[16]

Calculation of variations in the levels of HCMV viral load markers

Following the onset of antiviral therapy, variations in the levels of HCMV antigenemia, viremia, and DNAemia were expressed as percent variations, considering the relevant values observed on the day of starting antiviral therapy administration as 100%.

Statistical analysis

Differences between medians were compared by using Mann-Whitney U test for unpaired data or Wilcoxon's test for paired data. Differences in percentages were tested using Pearson's χ^2 test, while Fisher's exact test was used to evaluate differences in percentages when the total sample size was less than 30. The log-rank test was used to compare duration of treatment. All tests were two-tailed. Multivariate analysis was performed using a logistic regression procedure. Rising antigenemia, or the combined rise of antigenemia, viremia, and DNAemia were considered as independent variables. All dependent variables with a p value <0.1 in univariate analysis were included in the model of multivariate analysis, using a forward selection procedure. The probability of overall (OS) and event-free survival (EFS) was estimated by the Kaplan-Meier method and the significance of differences between curves was estimated by the log-rank test. Transplant-related mortality (TRM) was expressed as cumulative incidence curves, in order to adjust the analysis for competing risks.

Results

Given the similarity of the kinetics of different viral markers during treatment with ganciclovir in pediatric and adult HSCT recipients, only data relevant to pediatric patients are reported in Figures 1A to D.

Rising antigenemia in HSCT recipients

In group A, including 17 pediatric and 15 adult HSCT recipients, antigenemia initially rose significantly (p<0.001) from a median value of 2 (1-60) to a median peak value of 5 (1-130) pp65-positive PBL after a median of 7 (2-11) days of treatment, becoming negative after 25 to 30 days (Figures 1A and 2). In this group of patients, DNAemia and viremia started decreasing immediately after the onset of ganciclovir treatment, with a striking dissociation between the rising antigenemia and decreasing DNAemia and viremia. Median

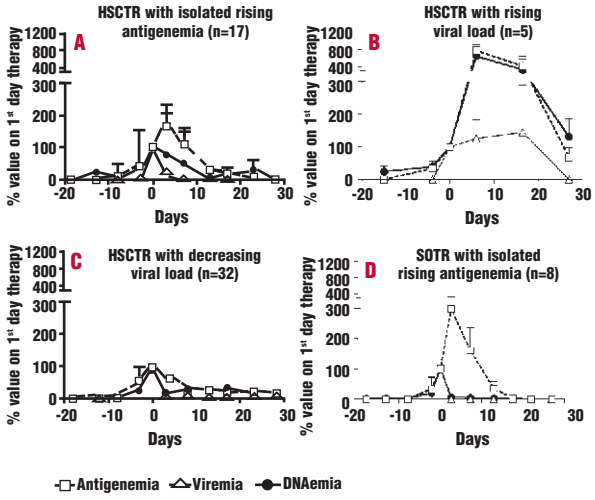

Figure 1. Mean kinetics of HCMV antigenemia, viremia and DNAemia in the following groups of transplanted patients showing varying levels of antigenemia during ganciclovir treatment: (A) group A: hematopoietic stem cell transplant recipients (HSCTR) showing rising antigenemia only; (B) group B: including HSCT recipients with rising whole viral load and steroid-treated GvHD; (C) group C: including HSCT recipients with decreasing viral load; and, finally, (D) group D: including solid organ transplant recipients (SOTR) with primary HCMV infection and rising antigenemia only. Day 0 indicates start of treatment, and values detected in the preceding or subsequent days are percentages of those found on day 0. Vertical bars indicate standard errors of the mean.

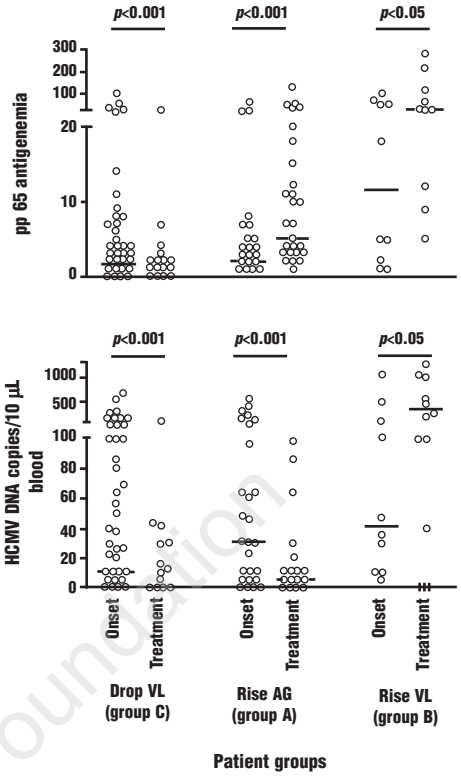

Figure 2. Median levels of antigenemia and DNAemia in the indicated 3 groups of HSCT recipients. Statistical analysis shows results of the comparison between values detected at the onset of treatment and peak values detected during treatment (groups A and B) or values detected after 7 days of treatment (group C). VL, viral load, AG, antigenemia.

DNAemia levels (5, 0-98 copies) at the time of the antigenemia peak were significantly lower ($p<0.001$) than those at the onset of treatment (30, 0-571 copies). This pattern of response to antiviral treatment was observed in 32/102 (31.4%) HSCT recipients of our series and, thus, was far from being a rare event. The median duration of treatment for this group was 29 (9-66) days, i.e. a median time of 29 days was required to achieve negative antigenemia results. This time was significantly longer ($p<0.001$) with respect to that of group C patients (median 16, range 5-42, days).

Rising viral load in HSCT recipients with GvHD

In a small number of HSCT recipients (group B) (10/102, 9.8%), rising antigenemia was associated with rising viremia and DNAemia, i.e. all markers of viral replication rapidly increased during ganciclovir treatment. This relatively infrequent pattern was observed in patients with grade II-IV acute GvHD, requiring treatment with high doses of steroids. In these patients, steroid therapy had been started at a median time of 4 (1-30) days prior to initiation of ganciclovir therapy. Antigenemia and DNAemia levels rose significantly ($p<0.05$) from median values of 12 (1-100) pp65-positive PBL and 46 (10-1,062) DNA copies at the onset of treatment to peak levels of 28 PBL (5-280) and 350 DNA copies (10-1,261), respectively. Peak levels of all viral markers were reached 8 (3-14) days after the start of antiviral treatment, negativization of all parameters being reached after 30 to 80 days (Figures 1B and 2). This type of response to antiviral treatment was similar to that observed in the presence of a ganciclovir-resistant HCMV strain. However, since no ganciclovir-resistant strain was detected in this group of patients, the rising viral load is likely to be due to lack of control of virus replication by the patient's immune system. In this adverse clinical situation, the approach adopted, whenever possible, was reduction of steroid therapy, either alone (6 patients) or together with adoption of a combination of 2 antiviral drugs (ganciclovir + foscarnet) (2 patients). Alternatively, when the same level of immunosuppressive therapy was maintained, the ganciclovir was replaced by foscarnet (2 patients). When viremia started to decrease or became negative, in parallel with viral DNA, antiviral treatment was considered to have been effective. The median duration of treatment of patients in group B was 59 (21-69) days, which was significantly longer ($p<0.001$) than that observed in both group C and group A patients.

Table 2. Risk factors for dissociated rising antigenemia or rise in whole viral load in HSCT recipients with HCMV infection treated with ganciclovir in the post-transplant period.

Risk factors	*Patient group*						*P*[b]	
	Decreasing viral load (group C)	*Rising antigenemia (group A)*	*OR (95% CI)*[a]	*P*[b] *(group A vs C)*	*Rising viral load (group B)*	*OR (95% CI)*[a]	*(group B vs C)*	
							UV	*MV*
Number of patients (%)	60 (58.8)	32 (31.4)			10 (9.8)		ns	na
Pediatric/adult patients	32/28	17/15	1.01 (0.4-2.4)	ns	5/5	1.14 (0.3-4.4)	ns	na
Recipient serostatus	57/3	30/2	1.27 (0.2-8.0)	ns	10/0	0.001 (0-30.9)	ns	na
Donor sibling/unrelated	41/19	16/16	2.16 (0.5-5.9)	ns	4/6	3.24 (0.7-9.3)	ns	na
HCMV+/HCMV-	45/15	21/11	1.57 (0.6-4.0)	ns	4/6	4.50 (1.1-18.1)	0.056	ns
Source of stem cells								
peripheral blood/bone marrow	22[c]/38	7/25	2.07 (0.6-6.7)	ns	2/8	1.44 (0.2-8.4)	ns	na
Anti-thymocyte globulin								
no/yes	54/6	27/5	1.67 (0.6-4.4)	ns	8/2	2.25 (0.4-6.2)	ns	na
HCMV infection								
onset after transplant (days)	24 (9-80)	25 (7-85)	na	ns	26 (11-41)	na	ns	na
antigenemia at onset[d]	1 (0-9)	1 (1-25)	na	ns	4 (1-54)	na	0.024	0.07
DNAemia at onset[e]	10 (0-500)	10 (0-268)	na	ns	38 (10-500)	na	0.050	ns
Antiviral treatment								
onset after transplantation (days)	36 (14-146)	34 (13-88)	na	ns	28 (14-50)	na	ns	na
onset after 1st HCMV detection (days)	7 (0-108)	5 (0-33)	na	ns	1 (0-11)	na	0.072	ns
antigenemia at onset of therapy[d]	2 (0-100)	2 (1-60)	na	ns	12 (1-100)	na	0.006	ns
DNAemia at onset of therapy[e]	10 (0-566)	30 (0-571)	na	ns	46 (10-1.062)	na	0.053	ns
No steroid/steroid treatment for GvHD	44/16	21/11	1.44 (0.6-4.2)	ns	0/10	56 (3-1021)	0.009	0.03

[a]OR, odds ratio (CI, confidence intervals); [b]ns: not significant (i.e. p>0.1); UV, univariate analysis; MV, multivariate analysis; na: not applicable; [c]: 1 cord blood; [d]no. pp65-positive PBL/2×10[5] examined; [e]no. HCMV DNA copies/10μL whole blood.

Viral load decreasing with treatment

In group C, comprising 60/102 (58.8%) HSCT patients, all viral markers started to decrease soon after the start of antiviral therapy (Figures 1C and 2). After a median of 7 (5-10) days of therapy (time selected for comparison with peak values of antigenemia or viral load of groups A and B), antigenemia (median 0, range 0-26, pp65-positive PBL) and DNAemia (median 0, range 0-144, DNA copies) levels were significantly (p<0.001) lower than at the onset of treatment (2, 0-100 pp65-positive PBL and 10, 0-566 DNA copies respectively). Treatment was continued until the antigenemia became negative in 2 consecutive tests performed 2 to 3 days apart. Thus, in this group, patients required significantly shorter treatment (p<0.001) than did patients in groups A and B. The median duration of positive antigenemia after the start of treatment was 16 (5-42) days.

Rising antigenemia in SOT recipients

For comparison, a group of 8 SOT (heart and/or lung) recipients treated with ganciclovir during primary HCMV infections was considered. These patients showed a pattern of response similar to that reported above for group A HSCT recipients. In more detail, the median level of antigenemia reached 298% of the level at the start of treatment after a median time of 5 days, then started to decrease. The level of antigenemia detected upon initiation of treatment was reached 10 days later, becoming negative between 15 and 20 days after the onset of therapy for most patients (Figure 1D). In contrast, DNAemia and viremia started to decrease immediately after the start of therapy.

Risk factors for rising antigenemia or rising viral load in HSCT recipients

In univariate analysis several potential risk factors for rising antigenemia or rising viral load were investigated: patient age at transplantation (pediatric vs adult patients), donor type (sibling vs unrelated donor), patient vs donor HCMV serologic status (seropositive vs seronegative), source of stem cells (peripheral blood vs bone marrow), use of serotherapy during conditioning regimen (ATG vs no ATG), time (days) to virus

Table 3. Incidence of HCMV recurrence, HCMV disease, transplant-related mortality, event-free survival and overall survival in the three groups of patients (univariate analysis).

Outcome	Patient group				
	Dropping viral load (group C)	Rising antigenemia (group A)	p[a] (group A vs C)	Rising viral load (group B)	p[a] (group B vs C)
HCMV recurrence	41/60 (68.3)	27/32 (84.4)	ns	7/10 (70.0)	ns
Treated HCMV recurrence	26/60 (43.3)	18/32 (56.3)	ns	7/10 (70.0)	ns
HCMV disease	0/60	0/32	ns	0/10	ns
Transplant-related mortality[b]	17% (9-31)	20% (10-42)	ns	30% (12-77)	ns
Event-free survival[c]	57% (43-72)	55% (36-74)	ns	30% (2-58)	0.03
Overall survival[c]	66% (53-80)	59% (40-79)	ns	40% (10-70)	0.06

[a]ns: not significant (i.e. p>0.1); [b]data are expressed as cumulative incidence and 95% confidence interval; [c]data are expressed as Kaplan-Meier probability and 95% confidence interval.

Table 4. Multivariate analysis (performed using the Cox proportional hazard regression model) comparing the influence of HCMV load and type of donor on transplant-related mortality, event-free survival and overall survival.

Parameter	Relative Risk	(95% CI)	p
Transplant-related mortality			
Unrelated vs sibling donor	5.17	(1.8-14.8)	0.0022
Group A vs group C	1.27	(0.4-3.6)	ns
Group B vs group C	1.70	(0.4-6.4)	ns
Event-free survival			
Unrelated vs sibling donor	2.90	(1.5-5.5)	0.001
Group A vs group C	1.18	(0.6-2.4)	ns
Group B vs group C	1.83	(0.8-4.4)	ns
Overall survival			
Unrelated vs sibling donor	3.86	(1.9-7.9)	0.0002
Group A vs group C	1.14	(0.5-2.4)	ns
Group B vs group C	1.62	(0.6-4.2)	ns

appearance in blood after transplantation by any test, levels of HCMV antigenemia and DNA at the onset of infection, time (days) to start of treatment after transplantation and after first virus detection in blood, levels of antigenemia and DNA at the start of treatment, and, finally, grade of acute GvHD (grade 0-I vs. II-IV), requirement or not steroid therapy (Table 2). Univariate analysis showed no difference between pediatric and adult patients with respect to the different parameters considered. Thus, in the following analysis, patients of the two age groups were examined as a single group. Univariate analysis showed that none of the parameters investigated was significantly associated with an isolated increase of antigenemia. Several parameters did, however, appear to be associated with rising viral load in univariate analysis, namely: HCMV seronegative donor, levels of antigenemia and DNAemia both at first detection in blood and at the start of treatment, early start of treatment with respect to virus detection in blood, and, finally, occurrence of grade II to IV GvHD requiring steroid treatment. However, in multivariate analysis, only occurrence of grade II-IV GvHD and, thus, use of steroids appeared to be a risk factor independently associated with rising viral load (Table 2).

Outcome in the three groups of HSCT recipients

The incidence of viral recurrence and progression to HCMV disease in the three groups of patients is reported in Table 3. No significant difference was found in the incidence of recurrence of HCMV infection in the three groups, and pre-emptive therapy was effective at preventing HCMV disease in all patients. We also evaluated the cumulative incidence of TRM, as well as Kaplan-Meier estimates of OS and EFS in the 3 groups. While in univariate analysis group B patients had lower probabilities of OS and EFS (*see Table 3*), in multivariate analysis these differences were not confirmed, transplantation from an unrelated donor being the only factor affecting TRM, OS and EFS unfavourably (Table 4).

Kinetics of viral markers after the start of treatment in individual HSCT recipients

Representative examples of the kinetics of HCMV viral markers after the start of treatment in individual patients from groups A to D are reported in Figures 3A to D. Figure 3B shows two peaks of viral load, one during treatment with ganciclovir and the second during treatment with foscarnet, which are the consequences of two courses of steroid therapy due to GvHD re-exacerbations.

Discussion

The results of the present study are mostly of interest for hematologists using antigenemia as the guiding assay for monitoring efficacy of pre-emptive therapy of HCMV infections in HSCT recipients. It was found that, on the average, in a population of both pediatric and adult patients, out of every 10 HSCT recipients receiving ganciclovir as pre-emptive therapy of HCMV infection, approximately 6 display declining levels of all viral markers immediately after the onset of therapy (group C), 3 show rising antigenemia in association with decreasing viremia and DNAemia (group A), and 1 shows increasing levels of all viral markers, in spite of ongoing antiviral therapy (group B). In view of these results, antigenemia can only be considered a surrogate marker of HCMV replication and does not reflect the

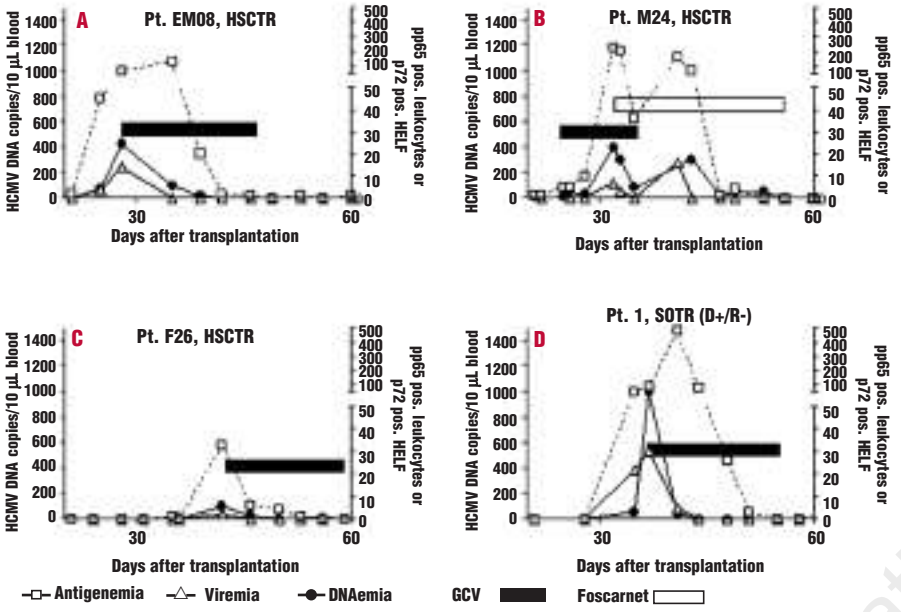

Figure 3. Kinetics of viral parameters of HCMV infection in individual representative patients from group A (A), group B (B), group C (C), and group D (D). While in (C) all viral markers drop after the start of therapy, in (A) and (D) only antigenemia is increasing, and in (B) all viral markers increase in two subsequent rejection episodes, both treated with steroids, while ganciclovir was administered during the first and foscarnet during the second episode. HSCTR: hematopoietic stem cell transplant recipients; SOTR: solid organ transplant recipients.

viral load, which is better represented by DNAemia and viremia. The clinical and therapeutic implication of this observation is that in patients of groups A (rising antigenemia) and C (decreasing viral load) the ongoing therapy with ganciclovir can be safely continued and a switch to an alternative drug or to combination therapy is unjustified. Whether some measures of therapeutic intervention (e.g. reduction of immunosuppressive therapy, when possible, or addition of foscarnet to ganciclovir) should be considered in group B patients (rising viral load) in order to obtain better control of viral infection and to prevent progression to HCMV disease, remains an issue to be further addressed.

The observation of rising antigenemia might prompt clinicians to shift from ganciclovir to foscarnet as antiviral therapy with the intent of overcoming problems related to the presence of a hypothetical ganciclovir-resistant strain. However, ganciclovir therapy can be safely continued in the presence of isolated rising antigenemia, provided that viremia (i.e. load of infectious virus in blood), and DNAemia (i.e. load of viral DNA in blood) are rapidly declining, thus documenting the effectiveness of antiviral therapy. Measuring the actual level of virus replication through determination of viremia and/or DNAemia offers the advantage of checking the effect of antigenemia-guided pre-emptive therapy. In this respect, although it would be desirable to perform all 3 viral assays, this is not the current practice. As a result, if antigenemia is adopted as the only guiding assay for deciding discontinuation or modification of pre-emptive therapy, one cannot differentiate between patients with isolated rising antigenemia, and patients with rising viral load. Thus, if a single test must be chosen for monitoring HCMV infection in HSCT recipients, determination of HCMV DNA load appears to be the test of choice to study the patients' response to treatment: determination of viremia, although reflecting actual viral replication, is a much less sensitive assay. In this respect, we are conducting a prospective, randomized clinical trial aimed at verifying whether the qualitative antigenemia cut-off so far used to guide pre-emptive therapy in our Department may be replaced by a quantitative DNAemia cut-off (a qualitative DNAemia cut-off has been used for several years in various transplantation Centers). A preliminary analysis shows that not only is adoption of a DNA cut-off safe, but also that this approach can spare a significant number of patients with spontaneous resolution of HCMV infection from being given antiviral treatment (*unpublished data*). Apart from other possible as yet unidentified factors, the interaction between the virus itself and ganciclovir seems to be the major factor responsible for isolated rising antigenemia during effective antiviral treatment. Using an *in vitro* model to study interactions between infected endothelial cells treated with different antiviral agents and polymorphonuclear leukocytes, we recently showed that the pathogenesis of the apparently paradoxical rise of antigenemia during antiviral treatment with ganciclovir is likely to depend on the fact that about 5% of infected endothelial cells escape the ganciclovir-induced block of viral DNA replication. Thus, large amounts of pp65 (dense bodies) are synthesized in a relatively small number of infected endothelial cells, from which pp65 is then transferred to polymorphonuclear leukocytes in increasing numbers.[17] The pheomenon in SOT recipients is likely to have the same pathogenetic basis. In addition, since isolated rising antigenemia in SOT

recipients has been observed to occur only in ganciclovir-treated primary HCMV infections, i.e. in HCMV-seronegative recipients of organs from seropositive donors, the presence of HCMV-specific immunity seems to be a condition preventing rising antigenemia. One can hypothesize that in HSCT recipients with an ablated immune system antigenemia rises during antiviral treatment in the absence of reconstituted specific immunity. A study aimed at verifying this hypothesis is warranted.

The results of our study indicate that, among potential risk factors responsible for a rise in whole viral load, only GvHD requiring steroid therapy appears to be an independent predictive factor. This finding suggests that steroid treatment enhance virus replication, probably through suppression of an HCMV-specific immune response. Thus, in HSCT recipients who have moderate to severe GvHD and a rise in viral load, proved by an increase of all viral markers, the immunosuppressive therapy should, whenever possible, be modulated, and antiviral therapy reconsidered. The major conclusion of this study is that, in the presence of isolated rising antigenemia or rising viral load in HSCT recipients treated with ganciclovir, one must not suspect, as a first-line probability, the presence of a drug-resistant HCMV strain, but rather identify the type of individual response to antiviral treatment and then decide therapeutic strategies accordingly. Ganciclovir-resistant HCMV strains have been mainly reported in patients given prolonged antiviral treatment with the same drug, mostly in association with an absent or delayed HCMV-specific T-cell-mediated immune response, and are less frequently encountered in HSCT recipients than in SOT patients.[18-21]

GG: conception, design, interpretation of results and draft of the article; DL: patient data collection and interpretation, database and statistical analysis; MZ: statistical analysis; EPA: adult patient follow-up; FB: molecular assays. MGR: antigenemia and viremia assays; FL: pediatric patient follow-up, data collection and interpretation. All authors reviewed the article critically and approved it for publication. The authors declare that they have no potential conflict of interest. We thank Linda D'Arrigo for revision of the English.

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