Efficacy of a viral load-based, risk-adapted, preemptive treatment strategy for prevention of Cytomegalovirus disease after hematopoietic cell transplantation

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Abstract

Cytomegalovirus (CMV) surveillance and preemptive therapy (PET) is the most commonly used strategy for CMV disease prevention in hematopoietic cell transplant (HCT) recipients. In 2007, we introduced a CMV prevention strategy for those patients at risk for CMV disease using quantitative PCR surveillance, with treatment thresholds determined by patient risk factors. Patients (N=384) received PET either at a plasma viral load of ≥500 copies/ml, at ≥100 copies/ml if receiving ≥ 1 mg/kg of prednisone or anti-T cell therapies, or if a ≥ 5-fold viral load increase from baseline was detected. Compared to patients prior to 2007 undergoing antigenemia-based surveillance (n=690) with PET initiated for any positive level, the risk-adapted PCR based strategy resulted in similar use of antiviral agents, and similar risks of CMV disease, toxicity and non-relapse mortality (NRM) in multivariable models. The cumulative incidence of CMV disease by day 100 was 5.2% in the PCR group compared to 5.8% in the antigenemia group (1 year: 9.1% PCR vs 9.6% antigenemia). Breakthrough CMV disease in the PCR group was predominantly in the gastrointestinal (GI) tract (16/20 cases, 80%). However, unlike CMV pneumonia, CMV GI disease was not associated with increased NRM (adjusted hazard ratio 1.13, P=0.8 [GI disease] vs. 8.41, P<0.001 [pneumonia]). Additionally, in this contemporary cohort CMV seropositivity in the donor or recipient was not associated with NRM at 1 year. Thus, the transition to a PET strategy based on CMV viral load and host risk factors successfully prevented CMV disease without increasing the proportion of patients receiving PET and attributable toxicity. Breakthrough disease in PCR-based PET occurs at a low incidence and presents primarily as GI disease which is more likely to be responsive to antiviral therapy.

Introduction

Strategies utilizing virologic surveillance and preemptive treatment have become the standard of care for the prevention of cytomegalovirus (CMV) disease after hematopoietic cell transplantation (HCT) [1,2]. However, a significant variation among transplant centers in testing methods, frequency, and thresholds for initiation of preemptive therapy remains [3,4]. As of 2003, nearly half of centers reported utilizing a surveillance strategy based on pp65 antigen in peripheral blood leukocytes, while the remainder had transitioned to a strategy based on plasma or whole blood CMV DNA level measured by polymerase chain reaction (PCR) [4]. Several cohort studies [5-12] and a few small randomized clinical trials [13-15] have compared the performance of these two tests for use in a preemptive treatment strategy. While pp65 antigenemia testing has been shown to perform well in CMV disease prevention, several operational disadvantages limit its use: the test requires circulating neutrophils and thus, is not reliable prior to engraftment; the samples require rapid processing to retain sensitivity; and interpretation of the slides requires highly trained personnel and has a high interobserver variability. In contrast, CMV DNA measurement by real time (RT)-PCR is more sensitive than pp65 antigenemia, provides more precise quantitation of CMV, can be automated, and is markedly less affected by specimen transport conditions and time [7-9].

In 2007, the Fred Hutchinson Cancer Research Center (FHCRC) changed from a preemptive strategy based on weekly surveillance of pp65 antigenemia to one based on CMV DNA-emia measured by quantitative RT-PCR. The strategy was designed with two primary goals: First, to ascertain a treatment threshold that was low enough to take advantage of the sensitivity of the PCR assay to identify early patients most likely to have a short doubling time [16] and those who progress to disease without high viral loads [7]. The second goal was to avoid increasing the overall proportion of patients treated to minimize adverse effects of therapy. We thus adjusted the viral load thresholds for preemptive treatment based on the patient's degree of immunosuppression, as a factor that correlates with viral replication dynamics [16]. Additionally, rapid relative increases of viral load were also chosen as an indication for preemptive treatment. In this study we report the efficacy of this risk-adapted, viral load-based strategy for the prevention of CMV disease after HCT and identify characteristics and outcome of breakthrough CMV disease with contemporary preemptive strategies.

Methods

Patient selection

The study included patients of all ages who were at risk of CMV disease and received their first allogeneic HCT at the FHCRC between 2002-2005 and 2007-2009. Patients at-risk for CMV disease were either CMV seropositive (R+; D- or D+) or seronegative patients receiving stem cells from seropositive donors (D+/R-). Patients receiving T-cell depleted stem cell products or umbilical cord blood transplants were excluded [17]. We excluded patients who underwent mixed CMV surveillance- sometimes tested by antigenemia, other times by PCR, during the transition period in early 2007 (n=127).

To control for secular trends in the frequency of secondary neutropenia and Gram negative bacteremia during the study time period we also analyzed patients who were CMV seronegative, received stem cells from a seronegative donor (D-/R-), and underwent first allogeneic bone marrow or peripheral blood stem cell transplant at FHCRC between 2002-2009. Because these patients continue to undergo antigenemia-based PET, they were not included in the analysis of CMV disease. This protocol was approved by the Institutional Review Board at the FHCRC.

Data sources

The FHCRC prospectively collects demographic, clinical and laboratory data from all patients undergoing HCT and the donors from the pre-transplant period through at least the first 100 days after transplant. Clinical and laboratory data after discharge from the center are also available from the long-term follow-up database; additional pathology, radiology and antiviral therapy data were extracted from the electronic medical record.

CMV surveillance, treatment, and antiviral prophylaxis

Patients at risk for CMV reactivation underwent weekly surveillance testing either by pp65 antigenemia or by PCR to measure plasma viral load. During the antigenemia era, surveillance testing was started after engraftment (after day 10) and continued weekly until day 100 [18]. As the PCR test does not require neutrophils, weekly surveillance was initiated about day 0. Patients who received preemptive therapy in the first 100 days or who were receiving steroids for chronic graft versus host disease (GVHD) continued

weekly PCR surveillance throughout the first year in both periods. Preemptive therapy with either induction-dose ganciclovir (5 mg/kg IV every 12 hours) or foscarnet (90 mg/kg every 12 hours; in case of neutropenia) was initiated for an antigenemia result of ≥1 positive cell per two slides [18] or, in the PCR era, for a CMV viral load ≥500 copies/ml or a 5-fold increase from baseline within previous month. Patients receiving anti-T cell therapies or ≥1mg/kg prednisone equivalent were treated at a viral load of ≥100 copies/ml. Induction dosing was continued for at least 7 days at which point, if antigenemia or plasma CMV viral load were decreasing, the therapy was changed to maintenance dose ganciclovir (5 mg/kg IV once daily) which would be continued for at least 2 weeks or until the repeat test was negative. After day 100, PCR surveillance was recommended in both cohorts with a preemptive treatment threshold of >=1000 copies/ml or a 5-times increase of viral load within one month. CMV plasma PCR was tested by a double primer assay as previously described [7]; the assay has a threshold of 50 copies/ml plasma.

Patients with CMV disease were treated with induction dose ganciclovir or foscarnet for 21 days, followed by maintenance dose for at least 3-4 weeks, or until day 100. Additionally, patients with CMV pneumonia were given CMV immunoglobulin (150 mg/kg) every other day for 14 days, then once weekly for the duration of maintenance treatment.

Acyclovir (250mg/m^2 IV or 800 mg orally twice daily) or valacyclovir (500 mg orally twice daily) was given to all patients for HSV-1, HSV-2 and VZV prophylaxis for at least one year [19]. No patients received high-dose acyclovir prophylaxis. All D+/R- and D-/R- patients received either CMV-seronegative or leukoreduced blood products until November 2009, at which time they received leukoreduced blood products only.

Other prophylaxis

Patients received levofloxacin (750mg once daily) or similar antibiotics during episodes of chemotherapy-induced neutropenia, trimethoprim/sulfamethoxazole or dapsone for *Pneumocystis jiroveci* prophylaxis [20], and fluconazole for prevention of candida infections [21]. Patients with a pre-transplant mold infection received an antifungal agent with mold activity in place of fluconazole. Conditioning regimens

and GVHD prophylaxis and treatment were performed according to center protocol as described elsewhere [22].

Outcomes and definitions

The primary outcome of this study was CMV disease in the first 100 days and 1 year post-transplant, which was classified according to standard definitions [23]. Secondary outcomes included: CMV reactivation defined as any positive surveillance test; non-relapse mortality (NRM) at 1 year post-transplant defined as all deaths not due to morphologic relapse of the underlying disease (in recipients of non-myeloablative conditioning NRM was defined as all deaths not due to morphologic relapse or progression of the underlying disease); CMV treatment-related neutropenia was defined as an absolute neutrophil count (ANC) of $<200/mm^3$ or $<500/mm^3$ in a patient who had previously achieved engraftment (ANC$>1000/mm^3$ for 3 days) within 60 days of initiating CMV therapy. The cultivation of any Gram-negative bacteria from the blood was considered an episode of Gram-negative bacteremia. Invasive fungal disease was classified according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) guidelines [24]; only “proven” and “probable” cases were included in these analyses.

Statistical Analysis

Patient characteristics were compared across cohorts using the Chi-square test. Univariate and multivariable competing risk regression models were used to calculate cumulative incidence estimates of outcomes detailed above [25]. Death was treated as a competing risk for all non-mortality outcomes. Relapse was treated as a competing risk for non-relapse mortality. Factors considered as potential confounders of the relationship between CMV surveillance and outcome were age, sex, donor age, sex mismatch, underlying disease risk (standard or high versus low), CMV serostatus, HSV-1 serostatus, conditioning regimen (myeloablative with high-dose total body irradiation (TBI), myeloablative without high-dose TBI, and non-myeloablative), HLA-matching (matched/related or unrelated/mismatched), and stem cell source (bone marrow versus peripheral blood stem cells). Post-transplant factors considered as

time-dependent covariates were: moderate to severe acute GVHD (grade 0-2 versus grade 3-4); chronic GVHD; and secondary neutropenia (ANC<500 after engraftment) [19,26-28]. All data were analyzed using Stata v11.2 (StataCorp, College Station, TX).

Because the CMV surveillance comparison groups were collinear with time, we could not determine whether differences in the incidence of secondary neutropenia or Gram-negative bacteremia that occurred over the study period were related to the CMV surveillance strategy without the introducing a third group of patients that spanned both eras but that did not undergo the same CMV surveillance strategy. Such a cohort was represented by the patients who were D-/R-. For these models, an interaction term between era and use of surveillance was utilized to test for the differential effect on the outcome of each CMV surveillance group as compared with D-/R- subjects within the same era.

All reported P-values are two-sided, calculated from the Wald test with values of less than 0.05 considered significant.

Results

The two CMV surveillance groups included 690 patients in the antigenemia group and 384 patients in the PCR group. The groups were similar with respect to patient age, sex, diagnosis, disease risk, HLA-matching, sex matching, donor age, and CMV donor/recipient serostatus (Table 1). Compared with the patients monitored by antigenemia, patients monitored by PCR were more likely to have received a nonmyeloablative conditioning regimen (P<0.001) and bone marrow as the stem cell source (P<0.001), and less likely to receive methotrexate- containing GVHD prophylaxis (P<0.001).

CMV disease. The incidence of CMV disease during the first year after HCT did not differ by surveillance method (Table 2 and Figures 1C and 1D). The probability of CMV disease among seropositive patients (R+) was 11.1% in the antigenemia group and 10.2% in the PCR group. There were only 5 cases of CMV disease in the D+/R- population (incidence 2.4% antigenemia vs. 3.2% PCR). HLA mismatch or unrelated donor was associated with increased risk of CMV disease, whereas the use of either a nonmyeloablative conditioning regimen or myeloablative without high-dose TBI were associated with a decreased

probability of CMV disease (Table 3). After adjusting for both of these factors, acute GVHD grades 3-4 and chronic GVHD also remained significantly associated with CMV disease.

Despite the absence of a significant difference in CMV disease overall with the PCR based surveillance strategy, the PCR- based surveillance strategy appeared associated with a lower risk of CMV pneumonitis (adjusted HR 0.56, 95% CI 0.3-1.1, P=0.07) but an increased risk of gastrointestinal disease (adjusted HR 1.43, 95% CI 0.8-2.6, P=0.25) after adjusting for conditioning regimen and HLA matching.

While the prevention of CMV disease was a primary goal in the design of our preemptive treatment strategy, a complementary goal was to limit treatment to those patients at highest risk of developing CMV disease. The probability of any CMV reactivation among R+ patients, was 59.0% (95% CI 54.8-62.9) in the antigenemia group and 75.7% (95% CI 70.9-80.3) in the PCR group. Among D+/R- patients the probability of any reactivation 23.4% (95% CI 16.4-31.1) in the antigenemia group and 33.9% (95% CI 21.1-43.9) in the PCR group. (Figure 1A) For the antigenemia group, any antigenemia was the treatment threshold, so that time to reactivation was the same as time to initiation of treatment. In the PCR group, however, the cumulative incidence of preemptive therapy among seropositive patients was 55.3% (95% CI 49.7-60.5) and was 25.8% (95% CI 15.7-37.1) in D+/R- patients. (Table 2) The selected risk-adapted CMV PCR treatment thresholds, while more sensitive, still appropriately restricted preemptive treatment such that the cumulative incidence of initiating preemptive antiviral therapy in the first 100 days post-transplant in the PCR group was not different from that of the antigenemia group (HR 0.96, 95% CI 0.8-1.1, P=0.7) (Figure 1, Panels A and B).

Table 1. Patient Characteristics

	CMV pp65 Antigen	CMV DNA PCR	CMV D-/R-
	n=690	n=384	n=621
	median (IQR)	median (IQR)	median (IQR)
Age in years	46.8 (35-56)	52.1 (35-61)	45.9 (30-58)
Donor age in years	40.1 (30-49)	37.2 (27-49)	36.1 (26-47)
	n (%)	n (%)	n (%)
Sex- Male	393 (57)	209 (54)	398 (64)
Disease- Acute Leukemia	322 (47)	160 (42)	269 (43)
Chronic Leukemia	83 (12)	50 (13)	76 (12)
Lymphoma	83 (12)	43 (11)	100 (16)
Other	202 (29)	131 (34)	176 (28)
Disease Risk*- Low risk	88 (13)	89 (23)	115 (19)
Standard risk	325 (47)	195 (51)	295 (48)
High risk	277 (40)	100 (26)	211 (34)
HSV-1 Serostatus**- Negative	130 (19)	64 (17)	131 (21)
Positive	560 (81)	318 (83)	474 (76)
Conditioning			
Myeloablative with high dose TBI^	150 (22)	60 (16)	146 (24)
Myeloablative without high dose TBI	327 (47)	151 (39)	283 (46)
Nonmyeloablative	213 (31)	173 (45)	192 (31)
HLA-matching- Matched/related	337 (49)	170 (44)	239 (38)
Mismatched/unrelated	353 (51)	214 (56)	382 (62)
Stem cell source- Bone Marrow	125 (18)	97 (25)	123 (20)
Peripheral Blood	565 (82)	287 (75)	498 (80)
Sex matching- Matched	364 (53)	203 (53)	340 (55)
Male donor to female recipient	152 (22)	88 (23)	123 (20)
Female donor to male recipient	174 (25)	93 (24)	158 (25)
CMV donor/recipient status- D+/R+	280 (41)	161 (42)	----
D-/R+	286 (41)	161 (42)	----
D+/R-	124 (18)	62 (16)	----
D-/R-	----	----	621 (100)
GVHD prophylaxis			
Calcineurin inhibitor alone	17 (3)	1 (<1)	7 (1)
Calcineurin inhibitor + MMF	232 (34)	116 (30)	190 (31)
Calcineurin inhibitor + MTX	394 (57)	107 (28)	300 (48)
Other***	47 (7)	160 (42)	124 (20)

Abbreviations: IQR= interquartile range, TBI= total body irradiation, R= recipient, D= donor, GVHD= graft versus host disease, MMF= mycophenolate mofetil, MTX=methotrexate

* Low risk disease includes chronic lymphocytic leukemia (CLL) in complete remission (CR), low grade Non-Hodgkin's lymphoma (NHL), high grade NHL in CR, Waldenstrom disease, multiple myeloma (MM) in CR, Acute lymphoblastic leukemia (ALL) in 1st CR, myeloproliferative disease, primary immunodeficiencies, and hemoglobinopathies. Standard risk disease includes refractory anemia (RA/RARS), CLL not in CR, MM not in CR, acute myeloid leukemia (AML) in CR, and chronic myeloid leukemia (CML) in 1st chronic phase (CP). High risk disease includes refractory anemia with excess blasts (RAEB/RAEB-t), AML evolved from myelodysplastic disease (MDS), high grade NHL not in CR, Hodgkin's disease, secondary MDS, AML not in CR, CML in 2nd CP or accelerated phase/blast crisis, non-hematologic malignancies, ALL not in 1st CR [41].

** These data are missing in 2 patients from PCR cohort and 16 patients in D-R-

^ high dose TBI= >12 Gy

*** Other includes data which were missing in 35 patients in the D-R- cohort

Table 2. CMV reactivation and disease endpoints stratified by CMV serostatus and surveillance method

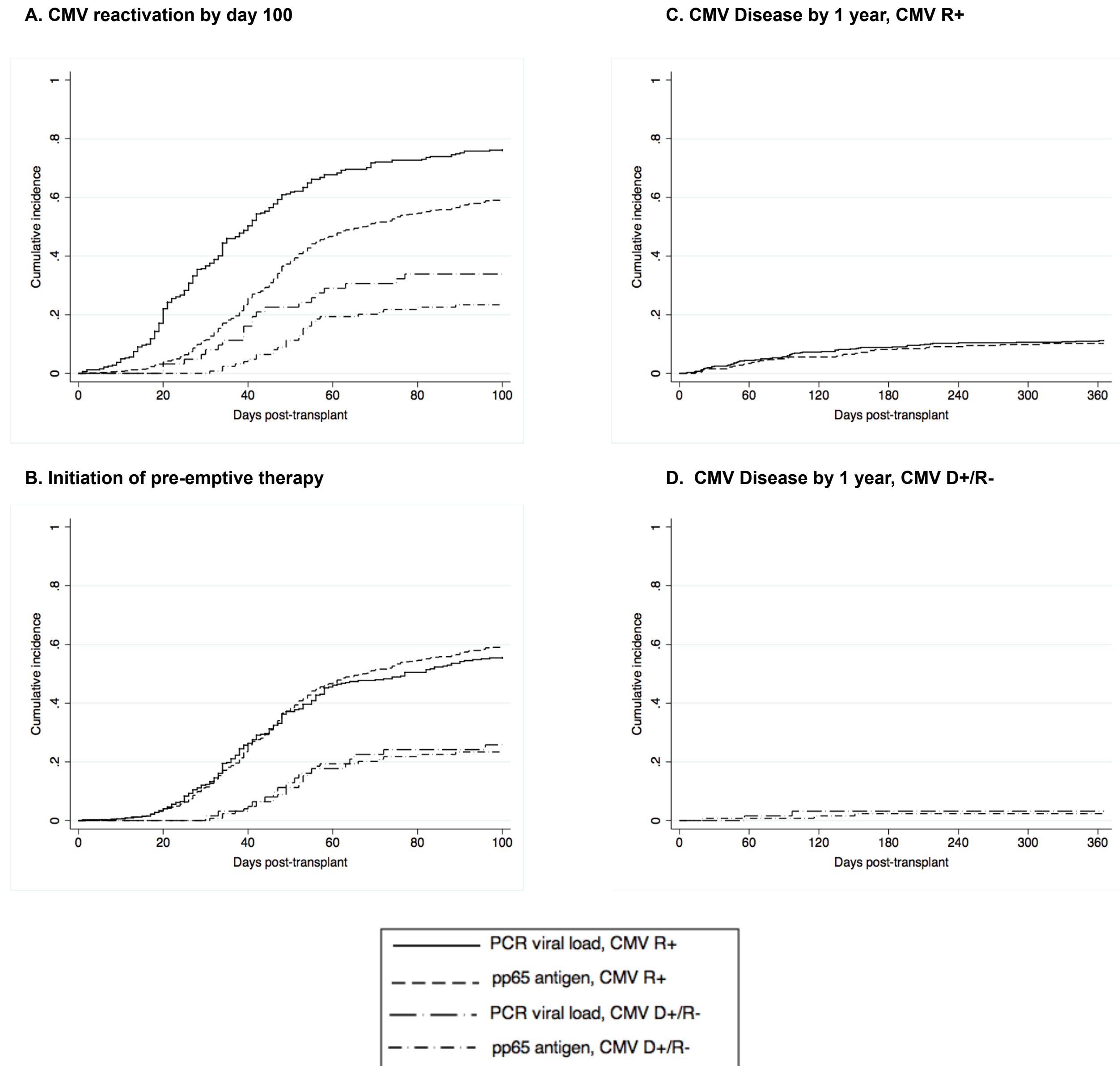
	CMV seropositive (R+)		CMV seronegative (D+/R-)	
	pp65 antigen (n=566)	**PCR viral load** (n=322)	**pp65 antigen** (n=124)	**PCR viral load** (n=62)
Any CMV reactivation by day 100				
Cumulative incidence estimate	59.0%	75.7%	23.4%	33.9%
Number of events	334	245	29	21
Median days to reactivation (range)	45 (2-97)	32 (0-97)	52 (31-89)	41 (20-77)
Initiation of preemptive therapy by day 100				
Cumulative incidence estimate	59.0%	55.3%	23.4%	25.8%
Number of events	334	178	29	16
Median days to initiation (range)	45 (2-97)	42 (1-97)	52 (31-89)	50 (30-96)
CMV disease by day 100				
Cumulative incidence estimate	6.9%	5.6%	0.8%	3.2%
Total number of events	41	18	1	2
Pneumonia	14	14	1	2
Gastrointestinal	27	4	0	0
Median days to disease (range)	47 (0-99)	52 (15-94)	19 (n/a)	77 (56,97)
CMV disease in first year				
Cumulative incidence estimate	11.1%	10.2%	2.4%	3.2%
Total number of events	64	32	3	2
Pneumonia	40	13	1	2
Gastrointestinal	24	19	2	0
Median days to disease (range)	89 (0-361)	88 (14-310)	116 (19-151)	77 (56-97)

Table 3. Risk factors for CMV disease by day 100 and one year post-transplantation

	CMV disease day 100						CMV disease 1 year					
	Univariate			Multivariable			Univariate			Multivariable		
	HR	95%CI	p value	adjusted* HR	95%CI	p value	HR	95%CI	p value	adjusted* HR	95%CI	p value
CMV surveillance- pp65 Antigen	1						1					
PCR	0.89	0.5-1.5	0.67	0.94	0.5-1.7	0.83	0.93	0.6-1.4	0.74	0.92	0.6-1.4	0.69
Age - < 40 years	1						1					
≥ 40 years	1.05	0.6-1.8	0.87				1.09	0.7-1.7	0.69			
Donor Age - < 40 years	1						1					
≥ 40 years	0.94	0.6-1.6	0.82				0.87	0.6-1.3	0.49			
Sex - Male	1						1					
Female	1.28	0.8-2.1	0.34				1.24	0.8-1.8	0.28			
Sex matching -- Matched	1						1					
Female donor/male recipient	1.38	0.8-2.5	0.28				1.1	0.7-1.8	0.69			
Male donor/female recipient	1.18	0.6-2.2	0.61				1.07	0.7-1.8	0.28			
Disease Risk - Low risk	1						1					
Standard risk	0.70	0.3-1.4	0.33				0.84	0.5-1.4	0.53			
High risk	1.13	0.6-2.3	0.73				1.07	0.6-1.9	0.81			
CMV serostatus - Negative	**1**						**1**					
Positive	**4.08**	**1.3-13.1**	**0.02**	**4.1**	**1.3-13.0**	**0.02**	**4.13**	**1.7-10.2**	**0.002**	**4.03**	**1.6-9.9**	**0.003**
HSV-1 serostatus- Negative	1						1					
Positive	1.1	0.6-2.2	0.77				0.94	0.6-1.5	0.94			
Conditioning regimen												
Myeloablative with TBI	**1**						**1**					
Myeloablative without TBI	**0.51**	**0.3-0.9**	**0.03**	**0.51**	**0.3-0.9**	**0.03**	**0.53**	**0.3-0.9**	**0.01**	**0.52**	**0.3-0.9**	**0.01**
Non-myeloablative	**0.41**	**0.2-0.8**	**0.01**	**0.42**	**0.2-0.8**	**0.01**	**0.62**	**0.4-1.0**	**0.06**	**0.63**	**0.4-1.0**	**0.07**
HLA-matching												
Related/matched	1						**1**					
Unrelated/mismatched	1.46	0.9-2.4	0.16	1.36	0.8-2.3	0.25	**1.7**	**1.1-2.6**	**0.01**	**1.62**	**1.1-2.4**	**0.02**
Stem cell source- Bone Marrow	1						1					
Peripheral Blood	0.93	0.5-1.7	0.82				1.04	0.6-1.7	0.89			
Acute GVHD - Grade 0-2	**1**						**1**					
Grade 3-4	**2.12**	**1.1-4.1**	**0.02**	**2.17**	**1.1-4.2**	**0.02**	**1.79**	**1.1-3.0**	**0.03**	**1.79**	**1.1-3.0**	**0.03**

* Hazard ratios are adjusted for CMV serostatus, conditioning regimen, HLA-matching. Acute GVHD was added to a model adjusted for CMV serostatus, conditioning regimen and HLA-matching.

Figure 1. Cumulative incidence of CMV reactivation (A), initiation of preemptive therapy (B), and CMV disease in seropositive (R+) recipients (C) and D+/R- patients (D), (n=1074)



Next, we compared the characteristics of the patients who developed CMV disease despite adherence to the prevention strategies. In the antigenemia cohort 33 of 41 cases of CMV disease in the first 100 days post-HCT (79%) occurred without a positive screening test (P<0.001). In the risk-adapted PCR cohort, only 4 of 20 cases (20%) occurred without a prior positive test (Table 4). Seven of the 20 cases (35%) in the PCR group were diagnosed after >4 days of pre-emptive therapy and all had gastrointestinal disease. In the PCR group, there were 9 cases (45%) where the PCR was positive at a level below the treatment

threshold so treatment was started less than 48 hours before CMV disease was diagnosed. In these cases, the median time from a first positive PCR test to CMV disease was 22 days (range 2-68 days). However, it is not clear that a lower treatment threshold would have prevented these cases. For example, patient G5 first showed evidence of CMV DNA-emia on day 20 post-HCT and preemptive therapy was initiated on day 27 for a rising viral load. She completed one week of induction therapy followed by two weeks of maintenance therapy. Her screening tests on days 56, 63, and 70 were negative and she was diagnosed with CMV gastritis on day 76 (Table 4). In fact, if the treatment threshold were lowered to any level of DNA-emia only 5 of these 9 patients would have received treatment earlier near the time of their CMV disease diagnosis. To lower the treatment threshold to any level of viral load would have meant treating an additional 67 patients (17% of the cohort) to possibly prevent 5 cases. If the treatment threshold were lowered to 100 copies/ml for all patients an additional 33 patients (9%) of patients would have been treated.

Late CMV disease (after day 100) occurred in 40 of the 897 patients who survived to day 100 without CMV disease. As the late CMV prevention strategy utilized PCR testing in both cohorts, differences between the antigenemia and PCR cohorts reflect only changes over time (early vs. late). While 18 of 26 patients (69%) in the early group had no PCR surveillance data within 30 days of their CMV disease, only 3/14 patients in the late group had not had surveillance testing within 30 days of their disease diagnosis. In the late group, 6/14 patients (43%, 4 cases pneumonia, 2 cases GI) had positive surveillance tests (viral load range 36-660 copies/ml) prior to their disease (median 24 days, range 7-26) but preemptive treatment was not started because the level was below the treatment threshold. The proportion of late CMV disease manifesting as pneumonia was similar in the two groups (9/14, 64% in PCR/early vs. 14/26, 54% in antigenemia/late, P=0.90).

Table 4. Characteristics of CMV disease cases occurring within the first 100 days post-HCT during PCR era

GI disease	Disease	CMV status	Day first test	Day first PCR +	Viral load (copies/ml) first PCR +	Day PET start	Day CMV disease	Days from PET start to CMV disease	Comments
Case									
G1	MDS	+	26	--	--	--	41	--	
G2	CMML	-	6	--	--	--	56	--	
G3	MM	+	4	27	330	--	20	--	
G4	ALL	+	6	12	34	--	66	--	PCR day 54 160 copies/ml, negative days 60 and 69
G5	AMOL	+	6	20	82	27	76	--	PCR negative days 56-77
G6	Other	+	8	15	34	--	83	--	PCR negative days 22-75
G7	CML	+	17	62	110	--	94	--	PCR negative day 90, 43 copies/ml on day 97
G8	MM	-	5	75	160	--	97	--	Persistent DNAemia (68-250 copies/ml) from day 75
G9	AMOL	+	16	16	83	19	21	2	
G10	ALL	+	20	41	6700	41	46	5	PCR negative days 20-34
G11	AMML	+	21	35	94	46	55	9	PCR negative days 21-28
G12	MM	+	28	83	140	83	93	10	PCR negative days 28-76
G13	MDS	+	12	38	1700	38	49	11	PCR negative day 27, next test delayed
G14	AML	+	0	26	300	29	43	14	
G15	AMML	+	19	47	260	51	69	18	
G16	CLL	+	19	30	57	39	62	23	
Pneumonia									
Case									
P1	AML	+	11	19	26	--	14	--	Concomitant fungal pneumonia prompted BAL
P2	NHL	+	11	41	56	--	56	--	PCR negative day 48, CMV diagnosed at autopsy
P3	ALL	+	17	17	100,000	17	19	2	First test delayed
P4	CML	+	12	18	67	21	22	1	

No positive screening test prior to CMV disease diagnosis

Preemptive therapy not initiated prior to CMV disease diagnosis or initiated within 48 hours of diagnosis

* all days are in reference to date of HCT (day 0)

Neutropenia. Despite similar rates of antiviral treatment initiation in the two CMV surveillance groups, patients monitored by PCR had a somewhat higher risk of ganciclovir- related neutropenia (ANC<200/mm^3) than those monitored by antigenemia (29.9% vs. 23.7%). Adjusting for age, use of MMF for GVHD prophylaxis, CD34+ cell dose (above or below the median), and relapse [27], patients in the PCR surveillance group had a 53% increased probability of ganciclovir-related neutropenia (adjusted HR 1.53, 95% CI 0.9-2.7, P=0.12). The two groups were similar in total days of ganciclovir or days of ganciclovir prior to this episode of significant neutropenia. The median days of ganciclovir use in the antigenemia group was 22 (range 9-55) days compared to the PCR group 21 (range 5-51) days (P=0.64). In an adjusted analysis of the entire study population including CMV D-/R- transplant recipients, secondary neutropenia, defined as any ANC<500/mm^3 after achieving engraftment and before day 100, was 3 times more likely to occur in the later era (2007-2009) than the earlier era (2002-2006) (HR 3.58, 95% CI 1.4-9.5, P=0.01). The cumulative incidence estimates were 20.8% (95% CI 18.4-23.3) in the early time period and 25.9% (95% CI 22.6-29.3) in the later period. To evaluate further whether the increase in neutropenia for the PCR group was caused by temporal changes in incidence, we tested the interaction between era and use of surveillance (P=0.20), indicating that the change in CMV surveillance strategy from antigenemia to viral load did not alter the risk of secondary neutropenia beyond the effects of time. Compared to the D-/R- subjects in the early time period (cumulative incidence 14.8%, 95% CI 11.3-18.8), the risk of secondary neutropenia in the antigenemia surveillance group (cumulative incidence 23.8%, 95% CI 20.7-27) was double (adjusted HR 2.15, 95% CI 0.9-5.2, P=0.09). In contrast, the adjusted hazard of neutropenia in the PCR surveillance group did not differ in D+/R- and R+ patients compared with D-/R- patients (cumulative incidence 29.9%, 95% CI 25.4-34.6 vs. 20.5%, 95% CI 16.0-25.3, HR= 1.01, 95% CI 0.5-2.1, P=0.9).

Invasive bacterial and fungal disease. Next, we aimed to, determine whether the trend towards more neutropenia led to more frequent invasive bacterial and fungal disease. We noted an increased risk of Gram-negative bacteremia within the first 100 days post-HCT in the PCR group (17.2%, 95% CI 13.6-21.1) compared to the antigenemia group (9.3%, 95%CI 7.3-11.6) (HR 1.90, 95% CI 1.3-2.7, P<0.001). However, an analysis including the D-/R- cohort and testing for interaction between surveillance use and era, illustrated that in comparison to D-/R- subjects within the same era (cumulative incidence 6.5% earlier vs. 12.0% later), the relative hazards of Gram-negative bacteremia was similar between those tested with PCR vs. antigenemia (P=0.83), suggesting that the

increased risk observed in the PCR group is confounded by time. The adjusted hazard ratio for the antigenemia group compared to the D-/R- patients was 1.44 (95% CI 0.9-2.3, P=0.14) and the adjusted HR for the PCR surveillance group was 1.34 (95% CI 0.9-2.0, P=0.16). Furthermore, the cumulative incidence of invasive fungal disease in the first 100 days after HCT was not significantly different between those patients in the antigenemia group (6.4%, 95% CI 4.7-8.4) compared to the PCR group (5.2%, 95% CI 3.3-7.8%) (HR 0.82, 95% CI 0.5-1.4, P=0.46).

Non-relapse and overall mortality. Non-relapse mortality in the first year post-transplant did not differ by CMV surveillance method. As depicted in Figure 2A, cumulative incidence of death without relapse in the antigenemia group was 24.2% (95% CI 20.9-27.9) at 1 year post-HCT, compared to 24.9% (95% CI 20.3-30.3) in the PCR group (P=0.76). In multivariable models, pre-transplant risk factors significantly associated with non-relapse mortality in this cohort were patient age >40 years, high risk underlying disease, and mismatched or unrelated HLA. CMV serostatus was not associated with increased NRM among the cohort at risk (R+ vs. D+/R-), nor when comparing to the D-/R- cohort (Table 5, Fig. 2B).

After adjusting for age, disease risk, and HLA-matching, post-transplant events associated with non-relapse mortality were grade 3-4 acute GVHD and several CMV related outcomes. Detection of any CMV reactivation, at any level by either method, was associated with a 57% increase in the probability of death and reactivation to levels above the treatment threshold was associated with a 81% increased probability. Controlling for neutropenia occurring after antiviral initiation diminishes the effect (adjusted HR 1.36, P=0.07), indicating that this increased risk of NRM may be mediated by treatment-related neutropenia. Finally, CMV end organ disease was associated with a 4-fold increased probability of death, most of this attributable to CMV pneumonitis because gastrointestinal disease was not associated with death (Table 5).

Overall mortality was also not different between the two surveillance groups after adjusting for age, disease risk, and HLA-matching (adjusted HR 0.94, 95% CI 0.8-1.2, P=0.76).

Table 5. CMV related risk factors for non-relapse mortality by 1 year post-transplantation (n=1074)

	Univariate			Multivariate		
	HR	**95%CI**	**p value**	**adjusted HR^**	**95%CI**	**p value**
CMV surveillance						
pp65 Antigen	1					
PCR	0.97	0.7-1.3	0.85	0.95	0.7-1.3	0.73
CMV serostatus						
D+/R-	1					
R+	1.16	0.8-1.7	0.41			
CMV serostatus*						
D-/R-	1			1		
D+/R-	1.04	0.7-1.5	0.83	1.10	0.7-1.6	0.62
D-/R+	1.33	1.0-1.7	0.04	1.29	1.0-1.7	0.07
D+/R+	1.1	0.8-1.5	0.49	1.13	0.8-1.5	0.4
CMV serostatus*						
D-/R-	1			1		
D+/R-	1.04	0.7-1.5	0.83	1.11	0.7-1.6	0.61
R+	1.22	1.0-1.5	0.10	1.21	1.0-1.5	0.12
Post-transplant risk factors#						
CMV Disease						
No	**1**					
Yes	**4.33**	**2.8-6.7**	**<0.001**	**4.28**	**2.8-6.6**	**<0.001**
CMV Gastrointestinal Disease						
No	1					
Yes	1.13	0.5-2.8	0.78	1.13	0.5-2.6	0.78
CMV Pneumonia						
No	**1**					
Yes	**8.62**	**5.2-14.4**	**<0.001**	**8.41**	**5.0-14.1**	**<0.001**
Any CMV reactivation						
No	**1**					
Yes	**1.66**	**1.2-2.2**	**0.001**	**1.57**	**1.2-2.1**	**0.003**
CMV reactivation triggering pre-emptive therapy						
No	**1**					
Yes	**1.87**	**1.4-2.5**	**<0.001**	**1.78**	**1.3-2.4**	**<0.001**
CMV reactivation triggering pre-emptive therapy, also controlling for post-treatment neutropenia				1.36	1.0-1.9	0.07

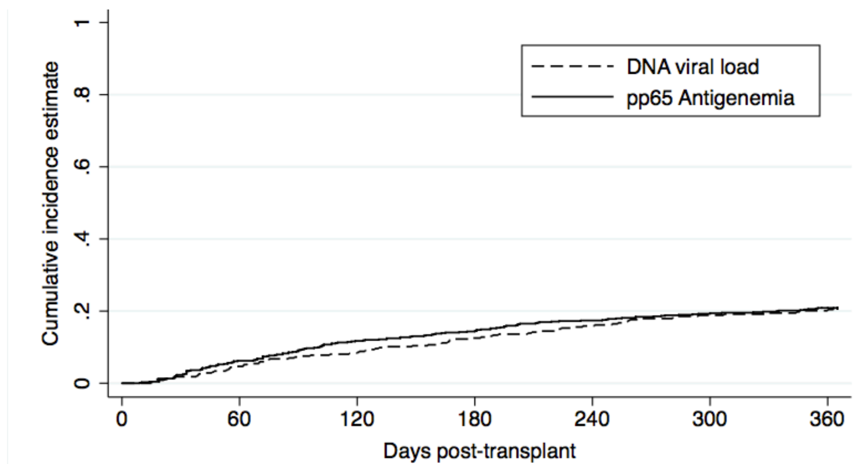
^ Hazard ratios are adjusted for age, disease risk, and HLA-matching

* Analysis includes original cohort plus 621 D-/R- patients

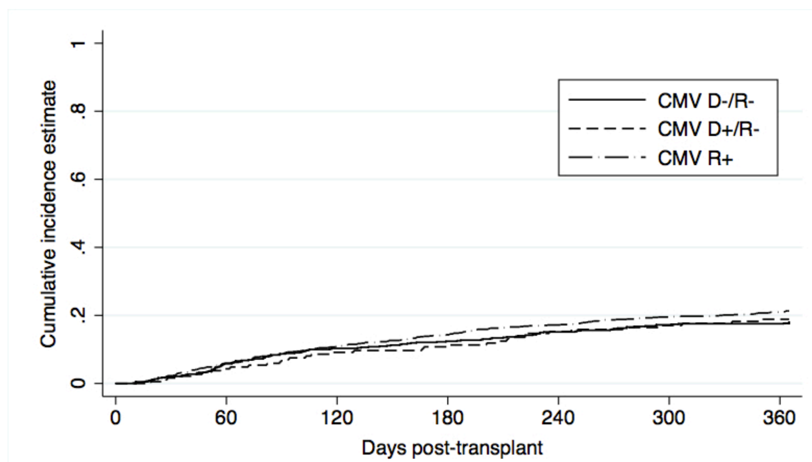
All post-transplant risk factors were individually added into a model adjusted for age, disease risk and HLA-matching

Figure 2. Cumulative incidence of non-relapse mortality by one year post-HCT

A. by CMV surveillance group (n=1074)



B. by Donor and Recipient CMV serostatus (n= 1695)



Discussion

In this prospective study of a large HCT cohort we describe the successful transition from a CMV surveillance strategy utilizing pp65 antigenemia testing to one using a quantitative PCR test with treatment thresholds determined based on patient risk factors. Our goal was to devise a prevention strategy that would be able to identify and treat those patients at highest risk of rapid CMV replication without increasing the proportion of patients receiving preemptive therapy and potentially exposing more patients to unnecessary toxicity. We observed no increase in initiation of preemptive therapy during the first 100 days post-HCT with the CMV PCR testing. In both surveillance groups, preemptive therapy was started in approximately 55% of the seropositive patients and 25% of

the seronegative patients. Furthermore, the incidence of CMV disease was similar between the surveillance groups with cumulative incidence estimates of ~10% in R+ patients and 3% in D+/R- by one year after HCT. These estimates, in general, are in agreement with other recent studies of CMV infection and disease after allogeneic HCT [28-31].

Interestingly, we found a trend toward decreased incidence of CMV pneumonia and increased incidence of gastrointestinal disease. Although these differences did not reach statistical significance, given the strong association between CMV pneumonitis and mortality, these trends may become clinically relevant. Few other studies report organ specific CMV incidence rates during this time period. In a smaller cohort of 186 patients receiving allogeneic transplants after reduced-intensity conditioning, Piñana and colleagues also describe a lower incidence of CMV pneumonia among patients followed by PCR surveillance than by antigenemia [29]. The fact that CMV gastrointestinal disease was not associated with overall mortality is noteworthy as it further supports that this manifestation of CMV disease is more amenable to treatment with antiviral therapy.

An important question to address is why we did not observe a reduction in gastrointestinal CMV disease in the PCR surveillance group. Our data seem to suggest that, unlike lung disease, CMV viral load in plasma does not adequately represent CMV replication in the gastrointestinal mucosa in some patients. Nearly half of the gastrointestinal disease cases occurred after the patient had been receiving induction therapy for at least 2 days indicating that treatment did not sufficiently halt replication to prevent disease or that replication in tissue had already started significantly earlier.

Late CMV disease continues to occur in approximately 4% of patients who survive to day 100. That 43% of late disease occurred among patients who had had multiple positive PCR tests just prior to their diagnosis, raises the question whether a lower threshold might be more effective in preventing late disease. This is especially concerning as more than half of late CMV disease cases are still pneumonia [32].

The PCR surveillance strategy was associated with an increased probability of ganciclovir-related neutropenia and gram-negative bacteremia, but the analysis suggests that the observed associations are confounded with time and may not be causally related to use of the PCR surveillance strategy. There was no association between use of PCR surveillance and increased risk of invasive fungal disease. A detailed examination of these findings was beyond the

scope of this paper but certainly calls for future exploration. In a prior analysis we noted a 50% increase in the rate of Gram-negative bacteremia in the first 100 days after allogeneic HCT as we transitioned from ceftazidime to levofloxacin for prophylaxis during neutropenia (2000-2002 vs. 2002-2005) [33].Other groups have reported an increasing risk of Gram-negative bacteremias after HCT associated with levofloxacin prophylaxis [34].

This study also suggested an association between PCR viral load-based preemptive therapy on non-relapse mortality. The association was diminished when we controlled for post-treatment neutropenia, but a statistical trend continued. Contrary to earlier cohorts [35], CMV donor and recipient pre-transplant serostatus was not significantly associated with non-relapse mortality at 1 year in this cohort. We speculate that this difference is due to improved prevention of fatal CMV disease, treatment-related toxicities and perhaps improved management of the indirect effects of CMV infection including invasive fungal disease [35,36]. Confirmation of this finding is other contemporary cohorts is needed.

Our study is limited by the observational design; we recognize that a randomized clinical trial comparing the two prevention strategies would have eliminated the problem of confounding with changing patterns over time. However, by adding the D-/R- cohort we were able to test for interaction between the surveillance method and time, and the large sample size and the uniform prevention strategy in each time period permitted us to perform multivariable modeling that likely accounted for possible confounders. The strategy evaluated here is the first that systematically combines quantitative plasma CMV testing with an ultrasensitive plasma PCR assay and in vivo replication dynamics to maximize early detection on the one hand and specificity on the other hand. Although such levels of sensitivity are not uniformly reached with contemporary commercial assays, newer assays with such a level of detection may soon become available [37,38]. Also, the availability of an international standard for CMV will make the results of this study more applicable [39].

In conclusion, the adoption of a viral load-based risk-adapted pre-emptive strategy for the prevention of CMV in a large allogeneic HCT cohort resulted in similar rates of CMV disease and non-relapse mortality without an increase in the proportion of patients receiving pre-emptive therapy or an attributable increase in the risk of ganciclovir-related toxicities. Setting a lower treatment threshold in those patients at greater risk of rapid CMV replication such as patients receiving high-dose steroids or anti-T cell therapies targeted pre-emptive treatment to those at greatest

risk. While CMV gastrointestinal disease continues to occur in the absence of DNA-emia, antiviral treatment is generally effective and there is no association with mortality. In contrast, PCR-based preemptive therapy effectively prevented CMV pneumonia, yet the few cases that did occur continued to be associated with extremely poor outcomes. Whether further lowering the threshold for preemptive therapy would be beneficial cannot be determined from this study. Our projections suggest that about 20-25% of the breakthrough cases could potentially have been prevented but at the expense of treating substantially more patients. The next truly innovative step forward in CMV prevention will probably only come with the availability of drugs with an improved safety profile that can be given either prophylactically or to a larger proportion of patients based on lower PCR levels or vaccines [40].

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