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ORIGINAL ARTICLE

Monitoring human cytomegalovirus infection in pediatric hematopoietic stem cell transplant recipients: using an affordable in-house qPCR assay for management of HCMV infection under limited resources

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Summary

Quantitative real-time PCR (qPCR) assay is accepted as the method of choice for monitoring human cytomegalovirus (HCMV) infection in hematopoietic stem cell transplant recipients, but the high cost of commercial kits has hampered its use in many developing countries. In this study, an affordable in-house qPCR was used to manage HCMV infection in pediatric patients and the diagnostic value of this method was compared with the conventional pp65 antigenemia assay. A total number of 1179 samples from 82 recipients were used in this study, and the effect of some potential risk factors on HCMV reactivation was evaluated. The qPCR was able to detect HCMV reactivation earlier and with higher sensitivity than antigenemia assay. Forty-six episodes of reactivation were detected in 39 patients, of which all were detected by the qPCR assay, while only 21 episodes were diagnosed by antigenemia. The DNAemia level of 1284 IU/ml plasma was defined as the optimal cutoff value for starting pre-emptive therapy. It was shown that the acute GVHD severity and the relationship of donor and recipient are the most significant risk factors for HCMV reactivation. The data suggest that the antigenemia method for monitoring HCMV reactivation could be substituted by the qPCR assay.

Introduction

Human cytomegalovirus (HCMV) infection remains a major cause of morbidity and mortality following allogenic hematopoietic stem cell transplantation (HSCT). The infection mainly results from reactivation of latent virus, but it can also be caused by primary infection [1,2]. The use of ganciclovir and some other antiviral drugs such as foscarnet and valganciclovir have reduced both the morbidity and mortality of HCMV disease [3–5]. Pre-emptive therapy strategy, to identify and treat only high-risk patients who have active HCMV infection prior to the onset of clinical disease, has been established as the treatment of choice for managing HCMV in transplanted patients [1,3,6]. This kind of therapy requires not only virus detection, but also determining whether HCMV is causing disease, because

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viremia may exists in many immunocompromised patients even in the absence of active disease. The most useful laboratory methods for pre-emptive therapy are those that quantitate HCMV, because greater quantity of virus correlates more with greater risk of HCMV disease [7]. The pp65 antigenemia and quantitative real-time PCR (qPCR) assays have been used mainly for HCMV pre-emptive therapy. Currently, qPCR is more accepted than pp65 antigenemia because it does not have some disadvantages of the antigenemia assay [7–9].

The main drawback of qPCR, which has hampered its general application in developing countries, is the higher cost of this method, especially when commercial assays are used. Therefore, the pre-emptive strategy for HCMV treatment in pediatric HSCT recipients at the Hematology-Oncology Research Center and Stem Cell Transplantation (HORCSCT) has been based on the results of antigenemia assay. A precisely validated and affordable "in-house" qPCR assay for quantitation of HCMV DNA in plasma samples that has been described previously can reduce the total cost of the HCMV monitoring procedure in patient recipients [10].

In this study, the qPCR assay was used to manage HCMV infection, and the diagnostic value of this method was compared to the pp65 antigenemia assay in a cohort study on pediatric patients. The effect of some potential risk factors on HCMV reactivation was also evaluated. Furthermore, it was tried to determine the DNAemia cutoff value of the qPCR assay and standardize the cutoff based on the WHO International Standard for Human Cytomegalovirus, for initiating anti-HCMV pre-emptive therapy.

Materials and methods

Patients and samples

From July 2011 to August 2012, a prospective cohort study was conducted on 82 pediatric patients (< 15 years old) who underwent allogenic HSCT at the Pediatric Transplantation Unit of HORCSCT. The patients were monitored for 120 days after transplantation by collecting blood samples once prior to initiation of conditioning regimen, twice a week from day 1 to 30, once a week from day 30 to 60, and every fortnight from day 60 to 120. About 5 ml of the blood samples were collected in two EDTA-anticoagulated tubes. One tube was processed for pp65 antigenemia, and the plasma portion of the other tube was used for DNA extraction and qPCR analysis. If an episode of HCMV infection was observed during the follow-up, the virological tests were performed twice a week until two consecutive negative results were obtained by both tests. The study was approved by the Institutional Review Board and ethics committee of HORCSCT, and all the parents signed a written informed consent.

HCMV treatment strategy

All patients received acyclovir prophylaxis (5 mg/kg intravenously three times a day) from conditioning until the day before transplantation. Ganciclovir pre-emptive therapy was initiated when ≥ 1 pp65 antigenemia-positive cells per 50 000 leukocytes were detected. Intravenous ganciclovir was administered (5 mg/kg twice a day) for at least three weeks or after two consecutive negative antigenemia results. The results of qPCR were not normally used for guiding pre-emptive therapy, except in situations that antigenemia-negative patients developed two consecutive high viral load (more than 1000 copies/ml) results along with the clinical symptoms of HCMV disease.

Definitions

Human cytomegalovirus infection was defined as the detection of the virus by antigenemia and/or qPCR tests, whereas episode of HCMV reactivation was defined either by a simultaneous positive result of antigenemia and qPCR, or two consecutive positive results of each test. Two consecutive negative results by both tests were defined the end of a given episode.

HCMV serology and pp65 antigenemia assay

The donor/recipient HCMV serostatus was determined before transplantation using the CMV IgM and CMV IgG electrochemiluminescence kits on an Elecsys 2010 analyzer (Roche Diagnostics, Mannheim, Germany) according to manufacturer‘s instruction. The pp65 antigenemia assay was performed using the CMV Brite Turbo kit (IQ Products, Groningen, the Netherlands), according to manufacturer‘s protocol. The cells were counted under a fluorescence microscope, and the results were expressed as the number of pp65-positive cells per 50 000 leukocytes.

HCMV DNA quantification

HCMV DNA was quantified using a validated in-house qPCR assay on the LightCycler®1.2 instrument (Roche Applied Science, Mannheim, Germany) as described previously [10]. The assay is based on hydrolysis probe technology and uses *avian Infectious Laryngotracheitis* (ILT) virus genome as internal control. The results were expressed as copy numbers of HCMV DNA per milliliter of plasma.

Statistical analysis

The correlation between qPCR and antigenemia was calculated using Spearman‘s rank correlation test. Days to the first positive qPCR and first positive antigenemia were ana-

lyzed by the Kaplan–Meier test, and differences in the two groups were assessed by the log-rank test. The Mann–Whitney *U*-test was used to compare medians between subgroups. The difference between groups was compared using the chi-square test. *P*-values of less than 0.05 were considered statistically significant. Receiver-operating characteristic (ROC) curve analysis and related area under the curve were performed to determine the optimal DNAemia cutoff value of the qPCR assay. All statistical analyses were calculated using SPSS software (version 16; SSPS Inc., 184 Chicago, IL, USA), but the Kaplan–Meier estimates were performed with Stata statistical software (version 6; Stata Corp., College Station, TX, USA).

Results

Patients

A total of 1179 consecutive samples were obtained from 82 pediatric patients. There were 28 (34.1%) female and 54 (65.9%) male, with a mean and median age of 7 year (range, 6 months to 14 years). Patients were monitored for a median of 108 days (range 18 to 198 days). A median of 15 samples per patients (range, 3 to 26 samples) were obtained. All donors and 81 of 82 recipients were HCMV IgG positive before transplantation, and one recipient who was IgM positive became IgG positive two weeks after transplantation. Other patient characteristics are detailed in Table 1.

Table 1. Baseline characteristics of patients.

Parameter	Number (%)
Gender	
Female	28 (34.1)
Male	54 (65.9)
Age	
Mean	7
Median	7
Std. Deviation	4.05
Minimum	6 months
Maximum	14 years
Underlying disease	
Beta-Thalassemia Major	29 (35.4)
Acute myeloid leukemia	18 (22)
Fanconi Anemia	7 (8.5)
Acute lymphoblastic leukemia	6 (7.3)
Osteopetrosis	5 (6.1)
Diamond blackfan Anemia	4 (4.9)
Leukocyte Adhesion Deficiency type I	2 (2.4)
Niemann-Pick disease	2 (2.4)
Wiskott–Aldrich syndrome	2 (2.4)
Acquired Severe Aplastic Anemia	1 (1.2)
Griscelli Syndrome	1 (1.2)
Metachromatic leukodystrophy	1 (1.2)
Mucopolysaccharidoses	1 (1.2)
Severe combined immunodeficiency	1 (1.2)
Sickle Cell Disease	1 (1.2)
Sickle-Thalassemia	1 (1.2)
Source of stem cell	
Bone marrow	32 (39)
Peripheral blood	47 (57.3)
Cord blood	3 (3.7)
Donor Type	
Sibling	57 (69.5)
Other relative	20 (24.4)
Unrelated	5 (6.1)
Acute GVHD	
None	40 (48.8)
Grade 1	9 (11)
Grade 2	9 (11)
Grade 3	14 (17)
Grade 4	10 (12.2)
ATG in conditioning regimen	
Yes	43 (52.4)
No	39 (47.6)

GVHD, graft-versus-host disease; ATG, anti-thymocyte globulin.

HCMV reactivation

Of the 1179 samples tested, 980 (83.1%) were negative by both antigenemia and qPCR assays. The remaining 199 samples, obtained from 51 patients, were positive by qPCR (110 samples, 55.3%), antigenemia (20 samples, 10%), or both (69 samples, 34.7%). The results of antigenemia and qPCR were discordant in 130 of the 1179 samples, but, as shown in Fig. 1, the overall results were correlated (r = 0.496, $P < 0.001$ by Spearman‘s rank correlation test).

The mean and median level of HCMV DNA load in antigenemia-positive samples were 7.3×10^3 and 626 copies/ml, respectively (range, 0 to 9.5×10^4), which significantly were higher than these values in antigenemia-negative samples that was 673 and 154 copies/ml, respectively (range, 0 to 1.4×10^4) ($P < 0.001$ by Mann–Whitney *U*-test).

The pattern of HCMV reactivation in patients were classified into six groups: (i) neither qPCR nor antigenemia was positive during follow-up (n = 31, 37.8%), (ii) qPCR alone was positive (n = 14, 17.1%), (iii) antigenemia alone was positive (n = 3, 3.7%), (iv) qPCR was positive earlier than antigenemia (n = 19, 23.2%), (v) antigenemia was positive earlier than qPCR (n = 6, 7.3%), and (vi) qPCR and antigenemia became positive simultaneously (n = 9, 11%). Of the 14 patients in group 2, 11 remained low viral load during follow-up by qPCR (median, 219 copies/ml; range, 65 to 398), whereas 3 patients had high viral load (median, 2188 copies/ml; range, 413 to 4331). These patients remained qPCR positive for more than three weeks. Whenever a discrepancy between the results of the two assays was observed in these patients, both assays were repeated again from sample preparation to results, but the patients still remained qPCR positive and antigenemia neg-

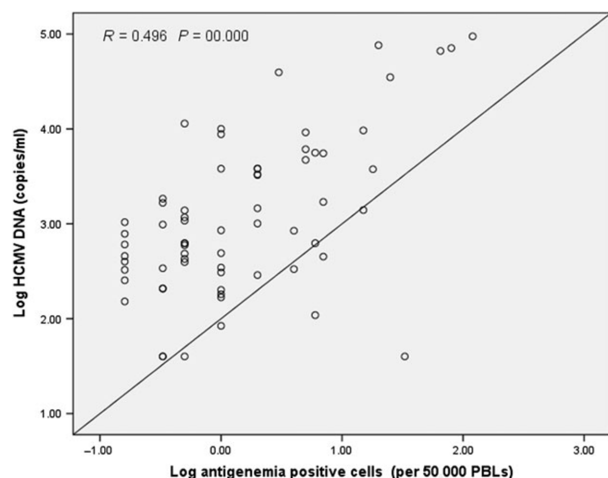

Figure 1 Correlation between the results of antigenemia and qPCR assays. Log10 DNA concentrations of HCMV DNA lever were plotted against Log10 number of pp65 antigenemia-positive cells per 50 000 leukocytes. The correlation coefficient and *P* value are shown in the upper left corner of the figure.

ative even when their samples were analyzed by another pp65 antigenemia detection kit (CINAkit CMV ppUL83, Argene, France). The absolute neutrophil count of these 3 patients was consistently more than 500 cells/μl throughout the follow-up, which was enough for antigenemia assay. Although pre-emptive therapy was guided after second positive result of qPCR in such discrepant cases, one of the patients died of pneumonia and GVHD (grade IV), 37 days after transplantation. On the other hand, there were three patients with positive antigenemia and negative qPCR results (group 3). All these patients showed viral reactivation only in one test and the quantity of the pp65 antigenemia-positive cells in their samples was always equal or less than one positive cell per 50 000 leukocytes.

Pre-emptive therapy and HCMV disease

Altogether 34 patients received ganciclovir pre-emptive therapy at least one time during the monitoring process because the evidence of HCMV reactivation at a median time of 52 days (range, 5–119) after transplantation. Four of 34 patients received treatment twice during the monitoring. Despite pre-emptive therapy, HCMV disease occurred in 8 (9.7%) patients from which 6 patients were treated successfully, but two died due to HCMV-related pneumonia.

Probability of positive antigenemia and qPCR

Based on Kaplan–Meier analysis, 37 (45.1%) of 82 patients developed positive antigenemia at a median of day 78 (range, 3 to 166), and 48 patients (58.5%) developed positive qPCR at a median of day 44 (range 3 to 152) (Fig. 2a and b). The difference between these two tests was statistically significant ($P < 0.001$ by log-rank test). The estimation was separately analyzed based on the relationship of donor and recipient, the grade of Graft-versus-host disease (GVHD) reaction, the underlying disease of the patients, and the presence of Anti-thymocyte globulin (ATG) in conditioning regimen (Table 2). Patients who received transplantation from sibling donors showed HCMV infection less frequently than patients who received transplantation from alternative donors, by both antigenemia and qPCR methods (Fig. 2c and d). On the other hand, patients with acute GVHD grade II-IV developed more reactivation than those with grade 0-I (Fig. 2e and f). When the incidence of HCMV reactivation was analyzed according to the presence of ATG in conditioning regimen, patients who had ATG in their regimen showed more reactivation than those without ATG by qPCR assay, but no statistical difference was observed based on the results of antigenemia assay. Finally, there were no difference between patients with leukemia and other patients (Table 2).

In another analysis, and to minimize the effect of false-positive results, episode of reactivation was considered. Altogether, 46 episodes of HCMV reactivation were observed in 39 patients. Table 3 summarizes all observed episode events. The qPCR assay was able to detect all 46 episodes, whereas only 21 episodes were detected by antigenemia assay and there was no case that antigenemia could detect episodes alone or prior to qPCR assay. There was a statistically significant difference between these two assays for episode detection ($P = 0.004$ by chi-square). The effect of previously described risk groups was assessed again based on the reactivation episodes, and the results were analyzed by chi-square or Fisher's exact test as appropriate (Table 4).

Determination of conversion factors between international units and copies

To make a conversion factor and express HCMV DNA results in standardized International Units (IU/mL), the WHO Standard (NIBSC code: 09/162; NIBSC, Hertfordshire, Britain) was used as the reference material [11]. Four dilutions of 10^5, 10^4, 10^3, and 500 IU/ml of the standard were prepared in human plasma known to be nonreactive for anti-HIV, anti-HCV, anti-HBsAg and negative for HCMV DNA. Each dilution was split into aliquots and analyzed on 3 different days on 4 replicates (12 replicates of each dilution). According to linear regression analysis, the conversion factor to convert copies/ml to IU/ml was calculated to be 1.07 (Fig. 3).

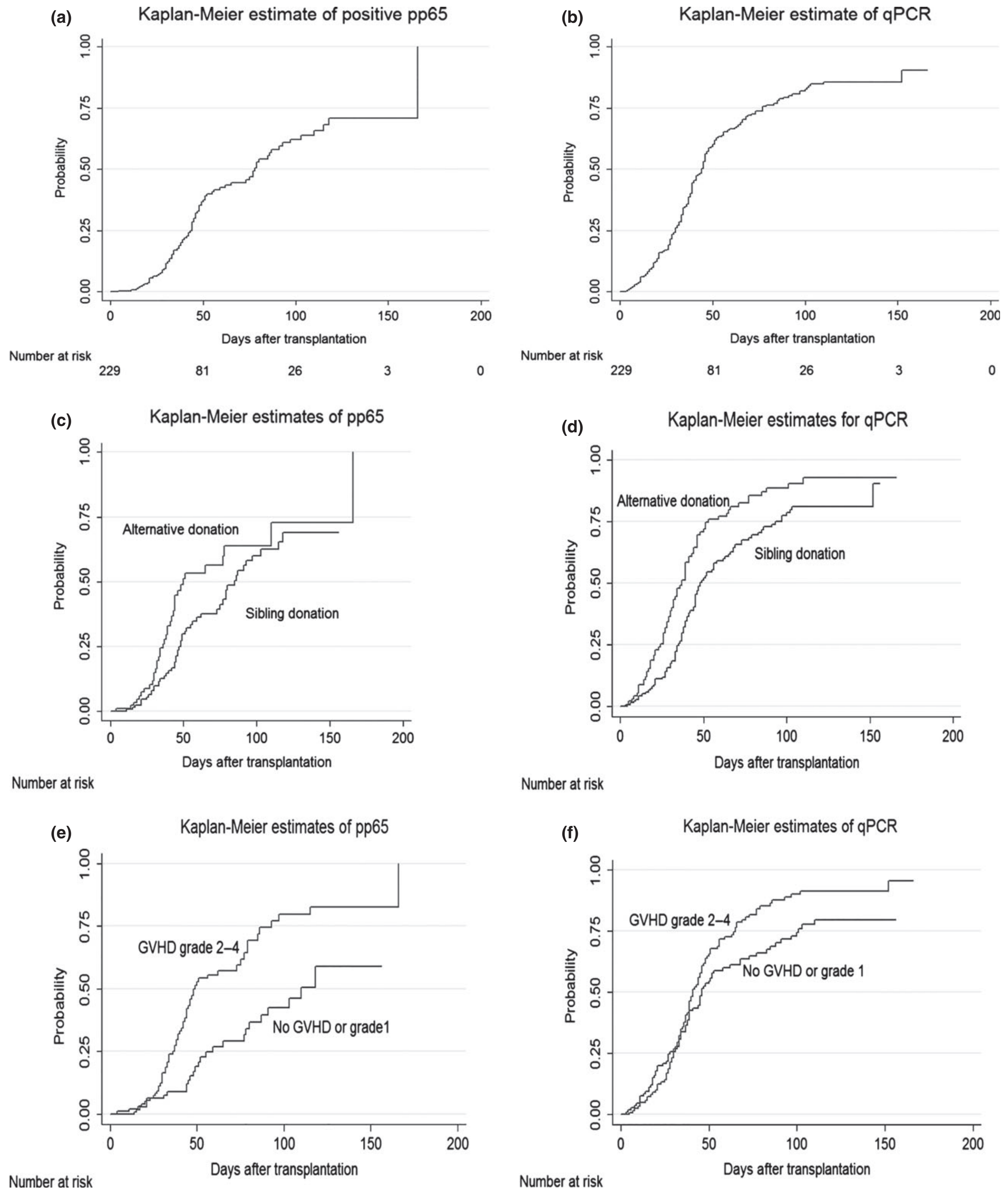

Figure 2 Kaplan–Meier plot of probability of HCMV reactivation. (a, b) Probability of developing HCMV reactivation by antigenemia and qPCR assays after transplantation ($P < 0.001$). (c) Probability of developing positive antigenemia in patients who received transplantation from sibling donors and the patients who received transplantation from alternative donors ($P = 0.030$). (d) Probability of developing positive qPCR in patients who received transplantation from sibling donors and the patients who received transplantation from alternative donors ($P = 0.011$). (e) Probability of developing positive antigenemia in patients with acute GVHD grade 0–I versus patients with GVHD grade II–IV. (f) Probability of developing positive qPCR in patients with acute GVHD grade 0–I versus patients with GVHD grade II–IV.

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Table 2. Longitudinal analysis of HCMV reactivation by antigenemia and qPCR assays in different groups of patients.

		Percentile (day)			
	Risk groups (patient number)	25th	50th	75th	*P*-value*
pp65 Antigenemia	Sibling donors (57)	48	85	0	0.030
	Alternative donors (26)	36	50	166	
	GVHD 0-I (49)	59	110	0	<0.001
	GVHD II–IV (33)	37	48	93	
	ATG in conditioning (43)	42	87	166	0.495
	Without ATG in conditioning (39)	44	77	118	
	Leukemia (24)	34	78	0	0.388
	Other disease (59)	45	79	166	
qPCR	Sibling donors (57)	35	48	93	<0.001
	Alternative donors (26)	24	37	53	
	GVHD 0-I (49)	32	47	85	0.018
	GVHD II–IV (33)	28	39	51	
	ATG in conditioning (43)	25	39	64	0.033
	Without ATG in conditioning (39)	36	48	86	
	Leukemia (24)	32	40	79	0.566
	Other disease (59)	27	45	73	

**P*-value for log-rank test.

Determination of DNAemia cutoff value

Receiver-operating characteristic (ROC) curve analysis was performed to determine the DNAemia cutoff value. The results of antigenemia assay were used as a reference standard to determine the optimal DNAemia cutoff value. The DNAemia cutoff was determined as the point with the maximum sum of sensitivity and specificity on the ROC curve using the Youden‘s index ([sensitivity + specificity]−1). Based on the results shown in Table 5 and Fig. 4, the calculated optimal DNAemia cutoff was 1200 copies/ml (area under curve: 0.788; sensitivity: 71%; specificity: 88%).

Costs-effectiveness of the in-house qPCR assay

In comparison with well-known, approved commercial qPCR kits that are used for HCMV quantitation, pp65 antigenemia assay has a lower cost. For instance, the price of a 110 test CMV Brite Turbo kit is about US$1000 (US$ 9.1 per test), while the price for a 96 tests artus® CMV LC PCR Kit (Qiagen, Hilden, Germany) is US$3100 (US$32.3 per test). Furthermore, the later assay requires DNA extraction step as well as other materials, which add additional cost to the qPCR test system. For instance, the additional costs per test for QIAamp DNA Mini Kit (Qiagen), LightCycler® Capillaries (Roche), and aerosol barrier pipette tips (Boeco, Germany) are US$4.4, US$0.77, and US$0.83, respectively. Therefore, the total cost of a representative commercial qPCR test would be about US$38.3, which is at least four times higher than the price of the antigenemia assay. Considering the fact that HSCT patients must be tested for HCMV reactivation several times after transplantation, this price difference was the main reason for not using qPCR assay for HCMV management in our center, especially because the cost of the assay had not been

Table 3. Quality of the reactivation episodes among patients.

Episode conditions	Patients (%)
No episode	43 (52.4)
one episode shown with qPCR	17 (20.7)
one episode shown by both methods	13 (15.9)
Two episodes shown by both methods	2 (2.4)
Two episodes shown first by qPCR and then by both methods	5 (6.1)
Two episodes shown first by qPCR and then by pp65	1 (1.2)
Two episodes shown only with qPCR	1 (1.2)

Table 4. The effect of the potential risk factors on episodes of reactivation.

Risk groups	*P*-value*
Sibling donors	0.053
Alternative Donors	
GVHD 0–I	0.004
GVHD II–IV	
ATG in conditioning	0.808
Without ATG in conditioning	
Leukemia	0.340
Other disease	

***P*-value for chi-square.

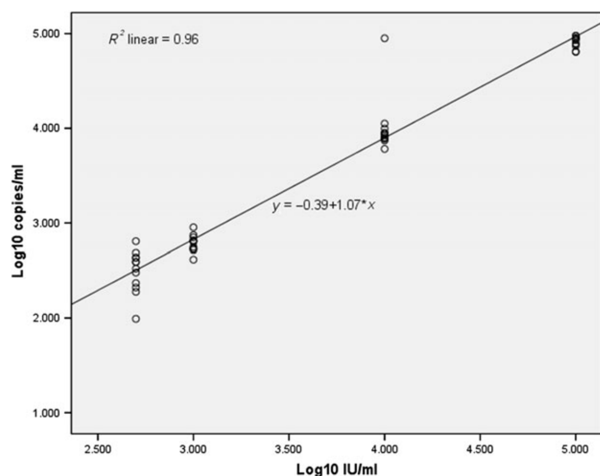

Figure 3 Relationship between HCMV DNA copies and International unit as measured by linear regression analysis. The R^2 value and linear equation are shown on the figure.

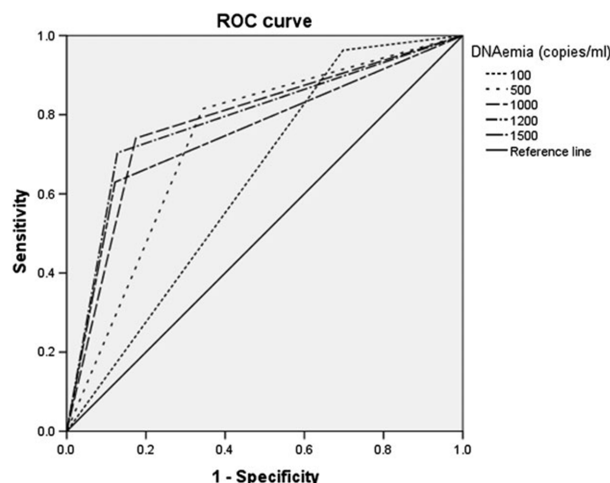

Figure 4 Area under the receiver-operating characteristic (ROC) curve corresponds to the data shown in Table 5. The antigenemia value of ≥ 2 pp65-positive cells/50 000 leukocytes were used for establishing the optimal DNAemia level cutoff.

Table 5. Performance of different levels of the HCMV DNAemia cutoff points.

DNAemia cutoff	Sensitivity (%)	Specificity (%)	Youden's index
100	96	30	0.26
500	81	65	0.46
1000	74	82	0.56
1200	71	88	0.59
1500	63	87	0.50

supported by the local insurance companies. On the other hand, the developed in-house qPCR assay is much more affordable and, as it is shown in Table 6, a single test using this assay would cost US$2.9(US$ 278.4 per 96 reactions), which is 1/11 of the price of well-known, approved qPCR kits. Nevertheless, when considering the costs of DNA extraction and other relevant materials, the price of the in-house qPCR test would be US$8.9 per test, which is as affordable as pp65 antigenemia assay. The cost-effectiveness of this validated in-house qPCR has resulted in using qPCR as the first option for HCMV monitoring in our center. Additionally, with this reduction in the price of qPCR assay, some insurance providers have accepted to cover the cost of the qPCR test.

Discussion

The serological data showed that almost all donors and recipients had anti-HCMV antibody before transplantation. This is similar to the results of other studies in the same region of the world [12–14]. The results of antigenemia and qPCR assays were discordant in 130 of the 1179 samples and 17 of the 82 patients, but the HCMV DNA load and the number of pp65-positive cells were correlated. Similar differences between the results of these two assays have been reported by other studies [6,9,15–20]. These discrepancies may be largely due to the higher sensitivity of the qPCR assay and the different natures of viral components that are diagnosed by these two assays (protein versus DNA). Based on the results of survival analysis in this study, qPCR was able to detect HCMV reactivation earlier and with higher sensitivity than antigenemia assay and there was no case that antigenemia could detect episodes alone or prior to the qPCR assay. As viremia is the most significant risk factor for HCMV disease [1], accurate and early diagnosis of HCMV reactivation could allow for timely intervention by pre-emptive therapy, thus reducing both the incidence and severity of HCMV disease [7,15].

There were three patients who had high DNAemia load for more than three weeks, without any positive result of antigenemia assay. In such situations, pre-emptive therapy was started after the second positive result of the qPCR assay because the first discordant observation could have been a false-positive result. However, this finding that the antigenemia assay did not become positive in some reactivated patients cannot be simply interpreted that the result of antigenemia assay does not become positive in some patients, and it requires further investigation.

When the incidence of HCMV reactivation was analyzed according to the potential risk factors, it was shown that the patients with GVDH grade II–IV developed more HCMV reactivation than patients with GVHD grade 0-I. These findings confirmed the results of many previous studies [16,21–25]. It was also shown that patients who

Table 6. Cost of the different ingredients that is used for the in-house qPCR assay.

Reagent	Supplier-Commercial name	Price based on US$	
		Per package (No. reactions)	Price per test
qPCR Master mix	Roche-LightCycler® TaqMan® Master	1010 (480)	2.1
HCMV and ILT Primers and Probes	Metabion	700 (1000)	0.7
Standard preparation	In-house	700 (>10000)	<0.1
DNA Extraction kit	Qiagen-QIAmp DNA Mini Kit	1100 (250)	4.4
qPCR vessels	Roche-LightCycler® Capillaries	372 (480)	0.77
Aerosol barrier pipette tips	Boeco-Top Line Natural Tip	8 (96)	0.83
Total			8.9

received transplantation from sibling donors developed reactivation less frequently than patients who received transplantation from alternative donors. A similar result has been reported by Yakushiji *et al.* in HSCT recipients [16]. This observation might be due to the fact that HLA compatibility between siblings is higher than either between the other relatives or unrelated donors. When the incidence of HCMV reactivation was analyzed according to the presence of ATG in conditioning regimen, patients who had ATG in their regimen showed more reactivation than those without ATG by qPCR assay, but no statistical difference was observed based on the results of antigenemia assay.

One of the main objectives of this study was to determine a DNAemia cutoff value for initiating pre-emptive therapy. As conventional pre-emptive strategy at HORCSCT has been based on the results of antigenemia assay, the results of this assay were used as a reference standard in the ROC curve analysis and the value of ≥ 2 positive cells was chosen to be the lower predictive antigenemia. Several HCMV DNAemia cutoff values, ranging from 200 to 20 000 copies/ml, have been previously reported in HSCT recipients [6,9,16,17,19,26]. This heterogeneity shows that there was no consensus DNAemia cutoff for starting HCMV pre-emptive therapy. This might be mostly because of the various variables that may influence the qPCR results. These variables include the sequence and the position of the primers and probes, the type of the probes, the quality of the qPCR master mixes, and the brand of the real-time PCR instrument. Therefore, the inconsistency of the cutoffs limits the ability of clinicians to compare values obtained from different laboratories. The availability of the first World Health Organization (WHO) international standard for *human cytomegalovirus*, NIBSC code 09/162 (CMV WHO Standard), has allowed developing conversion factor to convert copies per milliliter (copies/mL) to standardized international units per milliliter (IU/ml) [11]. However, to date, only few studies have reported a DNAemia cutoff that has been calibrated to the CMV WHO Standard [27–31]. At the beginning of the study, the results were reported HCMV concentrations in copies/ml because the WHO international standard was not available during development of the in-house qPCR assay. With the availability of the international standard, the relationship between copy number and international unit was examined later on in the course of the study and a DNAemia level of 1284 IU/ml was defined as the cutoff value for starting pre-emptive therapy. Therefore, during HCMV monitoring, antiviral therapy could be initiated if the DNAemia level exceeds this defined cutoff value. However, this DNAemia cutoff value is only applicable to pediatric patients who received HSCT.

On the other hand, the cost-effectiveness evaluation in this study showed that the price of the validated in-house qPCR method is as affordable as antigenemia assay, which is much more economical than the price of approved commercial qPCR kits. As in many developing countries, the most important contributing factor that defines the price of a laboratory test system might be the cost of materials, themselves and that the patients must be tested for HCMV reactivation several times during their follow-up, the affordability of the in-house qPCR assay helps to reduce the total cost of the HCMV monitoring procedure. Thus, the assay may be useful in situations where the high cost of commercial kits is a barrier for the application of qPCR. However, the pp65 antigenemia assay is still used in our as a backup or confirmatory assay.

In conclusion, the results of this study indicate that the in-house qPCR assay was able to detect HCMV reactivation earlier and with higher sensitivity than the conventional pp65 antigenemia assay. Our data also suggest that the acute GVHD severity and the relationship of donor and recipient are the most significant risk factors for HCMV reactivation.

Authorship

BK: performed the research and contributed for writing the paper. MP, YS and MM: contributed to the performance of the research, as well. AAH, HS and SMS: designed research,

prepared materials, and contributed for the writing of the paper. MK: collected and analyzed the data.

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