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# Preemptive therapy for cytomegalovirus based on real-time measurement of viral load in liver transplant recipients

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ABSTRACT

*Background:* Real-time PCR has emerged as the preferred diagnostic assay for CMV. However, its utility as a preemptive therapy tool for CMV disease and related outcomes in liver transplant recipients has not been fully defined.
*Methods:* Patients comprised 117 consecutive liver transplant recipients who underwent CMV surveillance monitoring using real-time PCR. Preemptive therapy with valganciclovir was employed upon detection of viremia. Baseline viral load was considered high based on log values (median).
*Results:* CMV viremia developed in 54% (63/117) of the patients, including 77% of R−/D+, 63% of R+/D+, 43% of R+/D−, and 10% of R−/D− patients. Overall, 23% (15/63) of the patients had recurrent viremia; R− serostatus ($p=0.065$) but not initial viral load correlated with recurrent viremia ($p=0.80$). At 12 months post-transplant, CMV disease occurred in 0.85% (1/117) of the patients (R+/D+recipient). None (0/30) of the R−/D+patients had CMV disease. Patients with CMV viremia treated preemptively did not differ significantly from those who never developed CMV viremia with regards to bacterial or fungal infections, rejection, graft loss, mortality rate, and probability of survival at 12 months ($p>0.05$ for all variables). The above outcomes also did not differ for patients with high (>1.9 logs) vs. low viral load (<1.9 logs) ($p>0.05$ for all outcomes).
*Conclusions:* Preemptive therapy guided by real-time PCR based monitoring led to outcomes in all patients or in those with high viral loads that were comparable to outcomes in patients who never developed viremia or had low viral loads, respectively. Late-onset CMV disease at 12 months was observed in <1% of all patients.


## 1. Introduction

Cytomegalovirus (CMV) is a significant opportunistic pathogen in organ transplant recipients [1]. Antigenemia based on the detection of structural tegument viral protein pp65 in the polymorphonuclear cells is an important diagnostic tool for CMV [2,3]. For nearly two decades, surveillance monitoring using antigenemia has been a widely used preemptive therapeutic approach for the prevention of CMV disease in hematopoietic stem cell and solid organ transplant recipients [4–7]. The test however, is labor intensive and is limited by the need for immediate processing, subjective bias in interpretation and quantification, and requirement of a minimal number of leukocytes in the peripheral blood [3]. Consequently, there is a growing trend towards replacement of antigenemia with molecular diagnostic assays [8–12].

Most laboratories performing quantitative nucleic acid testing for CMV are now using real-time polymerase chain reaction (PCR) based technologies which offer greater precision and accuracy, have broader dynamic range, and a faster turnaround time than conventional PCR assays [8,13]. To date, published experience documenting the clinical utility of real-time PCR tests for preemptive therapy exists largely in hematopoietic stem cell transplant recipients and in renal transplant recipients. [8–10,13–15]. A systematic and in depth analysis of outcomes with the use of real-time PCR guided preemptive therapy in liver transplant recipients has not been reported. The goals of this study are to report the performance characteristics of this assay and outcomes associated with its use as a preemptive therapy tool in liver transplant recipients, including high-risk recipient negative/donor positive subgroup.

## 2. Patients and methods

### 2.1. Patients

Beginning in August 2006, a quantitative real-time whole blood PCR assay replaced CMV antigenemia as a diagnostic test for CMV at our institution. Patients for this study therefore comprised consecutive liver transplant recipients at our center since August 2006 and

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who were followed until November 2009. All patients received tacrolimus and low-dose prednisone as the standard immunosuppressive regimen. Rejection episodes were treated with 1 g of methylprednisolone with or without a corticosteroid recycle (methylprednisolone administered in dosages tapered from 200 to 20 mg per day over 6 days). Perioperative antimicrobial prophylaxis consisted of ampicillin and cefotaxime administered for 24 h. All transplant recipients were administered trimethoprim-sulfamethoxazole daily indefinitely as prophylaxis for pneumocystosis.

## 2.2. Methods

Surveillance monitoring using real-time PCR was performed at weeks 2, 4, 6, 8, 12, and 16 after transplantation. The details of the real-time PCR assay that targets US17 and UL54 regions of the CMV genome have been reported elsewhere [11]. Upon detection of CMV viremia, preemptive therapy with valganciclovir 900 mg po bid was employed. Dosages were appropriately adjusted for renal dysfunction. A quantitative cut-off for CMV PCR was not used for the initiation of preemptive therapy. Valganciclovir was continued for a minimum of 21 days or until the PCR became negative. CMV disease included viral syndrome or tissue invasive disease defined using previously proposed criteria [7]. Tissue invasive disease required histopathologic evidence of CMV or positive immunohistochemical staining for CMV in tissue biopsy [16]. For the purpose of analysis, viral load in viremic patients was considered high based on i) log values greater than the median, and ii) log values greater than 75th percentile. Outcomes assessed included bacterial infections, invasive fungal infections, rejection, graft loss, mortality, and probability of survival at 12 months post-transplant. Bacterial infections and invasive fungal infections were defined as per criteria of the Centers of Disease Control and Prevention and as previously reported in liver transplant recipients [17,18]. The study was approved by the Institutional Review Board of the Pittsburgh VA Healthcare System.

### 2.3. Statistical analyses

Stata [version 10.1, College Station TX] was used for analyses. All CMV viral loads were log transformed. The Mann–Whitney rank sum test was used to compare viral loads between two groups. Categorical clinical data (presence of absence of rejection, graft loss and infections) were compared using the Chi-square or Fisher exact test. A Kaplan–Maier probability for 12 months survival was estimated and a log-rank test for equality of survivor functions was used to compare survival rates.

## 3. Results

### 3.1. CMV viremia and preemptive therapy

A total of 117 of 120 patients who underwent liver transplantation during the study period were considered evaluable since recipient or donor serostatus was equivocal in 3 patients. Of these, 26% (30/117) belonged to the R−/D+ group (Table 1). Overall, CMV viremia was documented in 53.8% (63/117) of the patients; these included 76.7% (23/30) of the R−/D+ patients, 63% (29/46) of the R+/D+, 42.9% (9/21) of the R+/D−, and 10% (2/20) of the R−/D− patients. R− patients undergoing primary infection had a higher viral load at baseline or the initial episode of viremia (median 276 copies/ml or 2.4 log copies/ml) compared to R+ recipients (median 66 copies/ml or 1.8 log copies/ml, $p=0.024$) (Table 1). Two weeks after initiation of valganciclovir, viral load declined by 0.368 log in R+ patients [−.475 to .944 log] whereas it increased by 0.261 log [1.28 to .849 log] in R− patients.

**Table 1**
Initial and recurrent viremia in study patients stratified by recipient and donor CMV serostatus.

| Recipient/donor CMV serostatus[a] | Viral load at baseline[b] | Recurrent viremia | Viral load at recurrence[2] |
|---|---|---|---|
| R−/D− ($n=2$) | 14,489 (333–28,646) | 50% (1/2) | 3015[c] |
| R−/D+ ($n=23$) | 276 (61–740) | 34.8% (8/23) | 470 (110–1633) |
| R+/D− ($n=9$) | 100 (67–385) | 11.1% (1/9) | 36[c] |
| R+/D+ ($n=29$) | 56 (23–217) | 17% (5/29) | 48 (21–313) |

[a]$n$ represents the number of patients in each group; [b]values represent median and interquartile (IQR) viral loads (copies/ml); and [c]IQR not applicable as only one patient in each group had recurrent viremia.

Recurrent viremia developed in 34.8% (8/23) of the R−/D+ patients, 17% (5/29) of the R+/D+, 11.1% (1/9) of the R+/D+, and in 1 of 2 R−/D− patients (Table 1). In all, 36% (9/25) of the R− and 15.8% (6/38) of the R+ patients had recurrent viremia ($p=0.065$). Viral load at initial viremia did not differ significantly for patients with recurrence (median 1.95 log, IQR 1.58–2.61 log) compared with those without recurrence (median 1.90 log, IQR 1.63 to 2.65 log, $p$-0.80). Valganciclovir was used for a median of 21 days [interquartile (IQR) 21–28 days] for the initial episode of viremia, and for 40.5 days (IQR 21–51 days) for recurrent viremia in patients with primary infection. In patients with reactivation infection, duration of valganciclovir for initial and recurrent viremia was median 21 days (IQR 21–21 days) and 32 days (IQR 21–42), respectively. Ganciclovir resistance was neither clinically suspected nor documented in any of the patients.

### 3.2. CMV disease and indirect outcomes

All patients were asymptomatic at the onset of viremia and none developed CMV disease during the surveillance monitoring period. Within 12 months post-transplant, CMV disease was documented in 0.85% (1/117) of the patients. The patient who belonged to R+/D+ group first developed CMV viremia 44 days after transplantation and received valganciclovir. Recurrent viremia with biopsy proven gastritis was diagnosed 7 months post-transplant. An additional patient (R+/D−) who never had CMV viremia documented during surveillance monitoring developed CMV disease>1-year after transplantation. Thus, a total of 1.7% (2/117) of the overall study population and none (0/30) of the R−/D+ patients had late-onset CMV disease at any time during the study period.

Patients with CMV viremia treated preemptively with valganciclovir ($n=63$) did not differ significantly from those who never developed CMV viremia during surveillance monitoring with regards to bacterial or fungal infections, allograft rejection, or graft loss due to retransplantation (Table 2). Mortality rate at 1 year (13.8% vs.12.7%, $p=0.85$) and the probability of survival at 12 months [0.839 (95% CI 0.71–0.91) vs. 0.842 (95% CI 0.69–0.92, $p=0.94$] did not differ significantly for patients with or without CMV viremia (Table 2). We sought to determine if any of the aforementioned indirect outcomes correlated with viral load in the overall study population, in patients with primary infection (R−) or in those with reactivation infection (R+). The median viral load for the patients with CMV viremia was 1.9 logs and patients were stratified into those with high versus low viral loads based on copies/ml greater than or less than the median (1.9 logs). No correlation could be shown for the indirect outcomes and high or low viral load (Table 2). Likewise, viral load>75th percentile (>2.5 logs) also did not correlate with outcomes (Table 2). In CMV seronegative recipients, an insignificant association was shown between high viral load (>2.5 logs) and the risk of bacterial infections but not other outcomes (Table 2). In R+ patients, high viral load (>2.5 logs) did not correlate with any of the indirect outcomes depicted in Table 2.

## 4. Discussions

Systematic monitoring with the use of a real-time PCR assay documented CMV infection (viremia) in 54% of our liver transplant recipients who received preemptive therapy. Previous studies in transplant recipients have documented CMV viremia based on real-time PCR assays in 52–66.6% of the patients [10,19]. Attempts at optimizing the predictive value of the test for CMV disease have led to the establishment of institutional thresholds or cut-offs as triggers for preemptive therapy. Viral load between 2000–5000 copies/ml in one study and between $10^{4.5}$–$10^{5.5}$ genomes/ml were considered optimal predictors of CMV disease in organ transplant recipients [20,21]. Other reports have documented 315 copies/$10^6$ cells as significant risk factors for CMV disease [22]. Thus, it is evident that generalization of particular cut-offs is limited by variations in performance characteristics of the test, assay design, and diversity in patient population studied. Additionally, there is evidence to show that high grade viremia but not detectable viremia per se correlates with poor post-transplant outcomes [14]. Since we aimed not only to prevent CMV disease but optimize outcomes, a quantitative cut-off was not used in our patients. It has therefore been our practice to treat CMV viremia preemptively, regardless of a particular threshold value.

We have previously shown that with the use of antigenemia for surveillance monitoring, preemptive therapy was required in 33.3% of