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Original Article

Clinical significance of human cytomegalovirus viruria and the effect of antiviral therapy in hematopoietic stem cell transplant recipients

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Abstract *Background:* Cytomegalovirus (CMV) causes life-threatening infections in immunocompromised host. The clinical significance of asymptomatic CMV viruria in patients receiving hematopoietic stem cell transplantation (HSCT) remains unclear. This study aims to clarify whether antiviral therapy is associated with a favorable clinical outcome.
Methods: HSCT recipients whose urine was culture-positive for CMV were retrospectively reviewed and followed. Viruria episodes were divided according to whether or not antiviral therapy was used. Mortality and the estimated glomerular filtration rate (eGFR) in 2 years following CMV viruria were compared between patients with and without antiviral therapy.
Results: Sixty-two episodes of culture-proven asymptomatic CMV viruria were identified in 28 HSCT recipients. Antiviral therapy was used in 35 (56.5%) and spared in 27 (43.5%) viruric episodes. Compared with the baselines, there were no significant difference in the decrements of eGFR between the two groups at the end the 1st year (4.78 vs 5.02 mL/min/1.73 m^2, $p = 0.968$) and the 2nd year (1.13 vs 7.66 mL/min/1.73 m^2, $p = 0.276$). Antiviral therapy for asymptomatic CMV viruria was also not associated with a favorable survival ($p = 0.288$). On the other hand, presence of CMV viremia correlated with a poorer survival (2-year mortality rate 60% vs 13.33%, $p < 0.001$).

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Conclusion: Antiviral therapy for asymptomatic CMV viruria is not associated with a clear clinical benefit in HSCT recipients. Further studies may be needed to identify if specific patient populations may benefit from antiviral therapy in CMV viruria.

Introduction

Human cytomegalovirus (CMV) is a herpes virus that ubiquitously infects human populations. The seroprevalence of CMV is around 60% in developed countries and may be even higher in Taiwan.[1,2] In primary infection, CMV generally produces asymptomatic or minimally symptomatic acute illness, such as infectious mononucleosis, in immunocompetent patients.[3,4] After the resolution of primary infection, CMV becomes quiescent and remains latent in myeloid cells.[5] When the host's immunity becomes compromised, CMV can reactivate and cause clinical disease, with infectious viral particles spreading via the bloodstream to many organ systems. A variety of life-threatening infections have been observed in immunocompromised hosts, including pneumonitis, colitis, retinitis, and encephalitis.[6]

The urogenital system may also be involved in CMV infection. The kidneys are among the main organs infected by CMV. In vitro, CMV readily infects kidney cells of glomerular, tubular, and vascular origin and induces signal changes associated with renal fibrosis.[7] In renal transplant recipients, CMV infection causes glomerular and interstitial damage, resulting in impairment of renal graft function and ultimately, graft failure.[8,9] Prophylactic or preemptive antiviral therapy in the presence of viuria or viremia are associated with a favorable outcome and can result in the preservation of renal function and lower incidence of graft loss in such patients.[10,11] However, although the importance of CMV viuria after kidney transplantation has been well documented, the clinical significance of CMV viuria in other types of transplantation is unclear.

Patients with hematologic diseases are at high risk for CMV disease. An early prospective surveillance study reported that the incidence of CMV infection in patients with acute leukemia ranged from 32% to 58%.[12,13] For patients receiving hematopoietic stem cell transplantation (HSCT), immunity first becomes compromised during the chemotherapy-induced neutropenic phase. Later, these patients become at risk for CMV disease owing to immune suppression therapy. In Taiwan, the incidences of CMV infection and clinically evident CMV disease in HSCT recipients are 45.3% and 6.8%, respectively.[14] The current guidelines indicate that HSCT patients should undergo regular blood examination for CMV and receive preemptive antiviral therapy in the presence of CMV viremia.[15] Nevertheless, the clinical significance of otherwise asymptomatic CMV viuria after HSCT is poorly understood. Furthermore, whether antiviral therapy in asymptomatic CMV viuria is beneficial remains uncertain. This study aims to clarify the clinical significance of asymptomatic CMV viuria in patients receiving HSCT. We investigated the association between antiviral therapy and patient survival as well as changes in renal function.

Materials and methods

Patients

This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (TVGH). Viral culture of urine samples taken from patients who had undergone HSCT was regularly performed every 4 weeks after transplantation. This was changed to a 2-week follow-up schedule in patients who were urine culture-positive for CMV. Adult patients (aged 20 years and older) who underwent either autologous or allogeneic HSCT at TVGH and who were urine culture-positive for CMV during the post-transplantation follow-up period were eligible for inclusion in the study. Patients were excluded if they were younger than 20 years old, pregnant, or had human immunodeficiency virus infection. For those included in the study, medical records were reviewed, and the clinical and laboratory data upon the first isolation of CMV from urine were recorded and followed up for 2 years.

Virology studies

CMV viuria was defined as CMV cultured from a urine sample. Clinical urine specimens were collected for viral culture at the Clinical Virology Laboratory of TVGH. The specimens were inoculated onto lung fibroblasts for viral replication. After visualization of the cytopathic effect, cells were stained with immunofluorescent antibody specific to CMV immediate protein (Dako, Denmark) to confirm CMV infection. For CMV quantitation in blood, viral DNA extraction and real-time PCR were performed using an automated DNA extraction and real-time PCR system according to the manufacturer's instructions (COBAS® AmpliPrep/COBAS® TaqMan® CMV Test; Roche Diagnostics, Mannheim, Germany).

Definitions and evaluations

The start of a CMV viuric episode was defined as obtaining a first positive CMV urine culture result; the end of the episode was defined as obtaining a negative CMV culture result. CMV viremia was defined as detection of more than 1000 viral copies per mL of blood by CMV real-time PCR. Asymptomatic viuria was defined if the patient had no fever and no urinary symptoms, such as hematuria, dysuria, frequency, or urgency, according to their medical records.

Collection and analysis of clinical data

We collected patient data including age, sex, underlying diseases, antiviral medication and immunosuppressive agents, serum creatinine level, urine CMV culture results, and blood CMV viral load. We also collected the cause of death for patients who had died. Episodes were divided into two groups according to whether patients received anti-CMV therapy, including valganciclovir and ganciclovir. Patients who received treatment were defined as group A, and those who did not receive treatment were defined as group B. The relationship between CMV infection and kidney function was established by analyzing the estimated glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology (CKD-EPI) equation in adults.[16]

Statistical analysis

Categorical variables were compared using the chi-squared test with Fisher's exact test. Continuous variables were compared using the *t*-test for data that followed a normal distribution and the Mann–Whitney *U*-test for data that did not follow a normal distribution. Survival was evaluated using the Kaplan–Meier method, and significant differences in survival curves were evaluated with the Mantel–Cox log-rank test. A value of $p < 0.05$ indicated a statistically significant difference. All analyses were carried out using IBM SPSS 22.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

Characteristics of patients with CMV viruria

Over the study period (2008–2014), 101 urine specimens from 28 patients were culture-positive for CMV, with 62 defined viruria episodes. Patient profiles are listed in Table 1. The most common underlying hematologic disease was acute myeloid leukemia, followed by non-Hodgkin lymphoma. Thirty-five (56.5%) viruria episodes from 20 patients treated with oral valganciclovir or intravenous ganciclovir (all under standard doses and adjusted according to body weight and renal function) were defined as group A, while twenty-seven (43.5%) viruria episodes from 11 patients during which the patients did not receive antiviral therapy were defined as group B. In group A, two patients were lost to follow-up in the second year. CMV viral load in blood was checked in 33 episodes, and 10 episodes had CMV viremia. In group B, blood CMV viral load was checked in 25 episodes and 4 episodes CMV viremia. The time gap between detection of viruria or viremia and initiation of antiviral therapy averaged 20.2 days (range 4.2–36.2 days). The mean duration of treatment was 44.05 days (range 19.05–69.05 days). There were also two patients in group B lost to follow-up. A total of 8 patients with 10 viruria episodes died within 2 years; their clinical profiles are listed in Table 2.

Table 1 Demographic and clinical characteristics of patients with cytomegalovirus viruria.

Characteristics	Overall patients, no		p
	Ever treated $n = 20$	No treatment $n = 8$	
Gender			
Male	13	5	0.901
Female	7	3	
Mean age (range)	44.09 (22–60)	44.38 (21–64)	0.908
Type of hematology diseases			
Lymphoma	7	2	0.609
Leukemia	6	6	
Multiple Myeloma	2	0	
Megakaryocytic hypoplasia	1	0	
Myelodysplastic syndromes	2	0	
Aplastic anemia	1	0	
Embryonal carcinoma of right testes	1*	0	
Time from identification of CMV viruria/viremia to treatment (mean ± SD days)	20.3 ± 16	N/A	
Duration of antiviral therapy (mean ± SD days)	44.05 ± 25	N/A	
Immune suppression medicine			
Nil	5	3	0.06
Cyclosporine	10	0	
Dexamethasone or prednisolone	6	5	

*The patient received hematopoietic stem cell transplantation.

Comparing the absolute GFR

Among group A patients, the eGFR at the time of CMV viruria detection was 94.28 ± 5.54 (mean ± SE) mL/min/1.73 m^2. The eGFR decreased to 89.50 ± 4.06 and 90.3 ± 4.5 mL/min/1.73 m^2 at the end of the first and second year after detection of CMV viruria, respectively. Both eGFR values were significantly decreased compared with the baseline, when CMV viruria was first detected ($p < 0.001$). Among patients in group B, the eGFR at the time of CMV viruria detection was 90.94 ± 3.59 mL/min/1.73 m^2; the values changed to 85.92 ± 5.86 and 86.07 ± 5.52 mL/min/1.73 m^2 at the end of the first and second year after detection of CMV viruria, respectively. Similarly to group A, both eGFR values after CMV viruria in group B patients were significantly decreased compared with the baseline ($p < 0.001$).

We then compared the decrements in eGFR between group A and group B patients. At the end of the first year after CMV viruria detection, similar decrements in eGFR were observed in group A and group B patients (4.78 and 5.02 mL/min/1.73 m^2, respectively; $p = 0.968$). At the end of the second year after CMV viruria detection, a slightly greater decrement in eGFR was observed in group A compared with group B (1.13 vs. 7.66 mL/min/1.73 m^2) but the changes were not statistically significant ($p = 0.276$; Fig. 1). The mean percentages of decrements

Table 2 Demographic and clinical characteristics of expired patients.

Case no.	Age/gender	Underline disease	Antiviral treatment (appropriate/no treatment/inadequate dose)	Days from CMV viruria or viremia to treatment	CMV viremia	Immune suppression medicine	Co-morbidity	Cause of death
1	56/F	Aplastic anemia	First episode: Yes Second episode: No	2	First episode: Yes Second episode: Yes	Cyclosporine	GVHD Chronic HBV hepatitis	Diffuse large B cell lymphoma
2	51/M	AML	First episode: No Second episode: Yes	14	First episode: Yes Second episode: Yes	Cyclosporine	GVHD HBV carrier Interstitial pneumonitis	Invasive pulmonary aspergillosis
3	59/M	AML	No	N/A	Yes	Prednisolone	GVHD	OHCA
4	42/F	ALL	No	N/A	No	Prednisolone	GVHD	Intracerebral hemorrhage
5	64/M	AML	No	N/A	N/A	Prednisolone	GVHD COPD	Pneumonia
6	47/M	Multiple myeloma	Yes	30	Yes	Prednisolone	Nil	Pneumocystis jiroveci pneumonia
7	51/F	AML	Yes	7	Yes	Dexamethasone	Mixed hyperlipidemia	Pneumonia
8	59/M	MDS	Yes	16	Yes	Cyclosporine	GVHD Decompensated heart failure Gout	Pneumonia

AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; CMV, cytomegalovirus; GVHD, graft versus host disease.

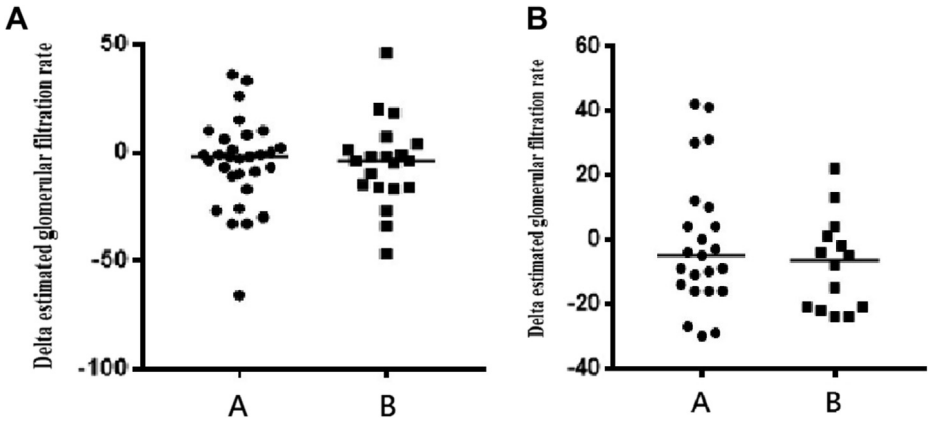

Figure 1. Comparison of the changes in estimated glomerular filtration rate (eGFR) 1 year (1a) and 2 years (1b) after presence of cytomegalovirus viruria. Group A: viruria episodes during which the patients received antiviral therapy; group B: episodes during which patients did not receive antiviral therapy.

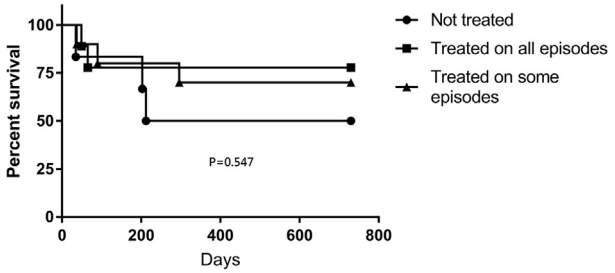

Figure 2. Comparation of survival between patients with or without antiviral therapy. Not treated: patients who never received antiviral medicine treatment; treated on all episodes: patients who received antiviral therapy in every episode; treated on some episodes: patients who received antiviral medicine treatment in some episodes.

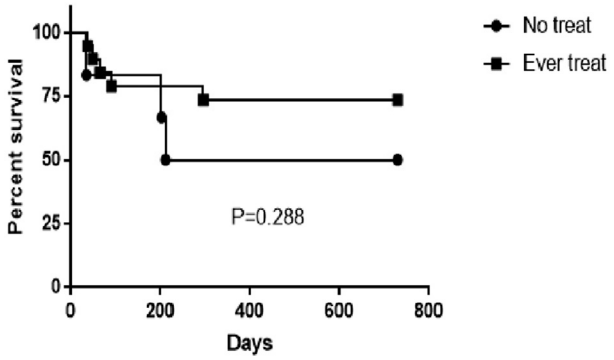

Figure 3. Comparation of survival between patients with or without antiviral therapy. Not treated: patients who never received antiviral therapy; ever treated: patients who received antiviral therapy in all or some episodes.

at the end of the first and second year were 0.4% and 5.6% in group A and group B, respectively ($p = 0.822$); those at the end of the second year were 3.4% and 8.6%, respectively ($p = 0.175$).

Comparison of survivals between patients with and without antiviral therapy

Among 28 patients, 3 were lost to follow-up. Nine (36%) patients received antiviral treatment during every episode, 10 (40%) patients received occasional treatment, and 6 (24%) patients received no antiviral treatment during any episode. The 2-year survival rates were 77.8%, 70%, and 50% in these three patient groups, respectively ($p = 0.547$; Fig. 2). For patients who had ever received antiviral therapy and those who never received antiviral therapy, the 2-year survival rates were 73.68% and 50%, respectively. The Kaplan–Meier curves indicated that there were no significant differences in post-viruria survival rates regardless of whether the patients received antiviral therapy ($p = 0.288$; Fig. 3).

Viremia and the outcome of patients

Blood CMV viral loads were determined in 25 of the 28 patients with viruria. Blood viral loads over 1000 copies/mL were detected in 10 (40%) of the 25 patients. Among these, 6 (60%) patients died within the 2-year follow-up period. In contrast to the high mortality rate in patients with CMV viremia, only 2 of 15 (13.3%) patients without CMV viremia died during the follow-up period. The Kaplan–Meier curves indicated that the presence of CMV viremia was associated with poorer survival in patients with only CMV viruria ($p < 0.001$; Fig. 4). Only two patients with viremia did not receive antiviral treatment and they were both expired (Table 2, case No. 3 and 4). Eight patients received antiviral treatment, and four (50%) patients expired.

Discussion

CMV is one of the most important factors that can affect the survival of patients receiving HSCT. In HSCT recipients, infection by CMV is associated with high rates of mortality and morbidity.[17] Several organ systems may be involved and infections may include pneumonitis, retinitis, and gastroenteritis or colitis. Although CMV has been reported to cause nephritis and cystitis in HSCT patients, the exact influence of CMV infection in the urogenital tract has seldom been assessed. In this study, we aimed to clarify the

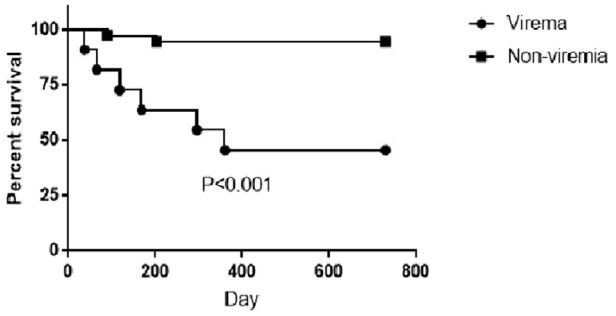

Figure 4. Comparation of survival between patients with or without CMV viremia.

clinical significance of asymptomatic CMV viruria in HSCT recipients through analyzing the effect of antiviral therapy in episodes of viruria. We found that only some patients with CMV viruria received antiviral treatment, reflecting the fact that the presence of CMV viruria is frequently considered of little consequence by clinicians.

Most of the patients with CMV viruria in this study had a significant decrease in eGFR after transplantation. However, the cause of deteriorating renal function can likely be attributed to underlying hematologic diseases or HSCT itself.[18] Even though antiviral therapy for CMV viruria was associated with a slight trend towards a slight decrease in eGFR, no clear differences could be identified between our two patient groups with regard to survival rates and preservation of renal function. One reason is that most of the patients in our study received antiviral treatment too late. Unlike serum antigen or antibody tests, the results of which can be obtained immediately, it usually takes days or even weeks for CMV to be isolated from cell culture.[19] A treatment strategy based on detection of CMV through the convention cell culture system may not be timely enough to prevent tissue damage caused by the virus. The other reason may be that CMV can infect the urinary tract without damaging the kidneys. Although nephritis is among the spectrum of urinary infections caused by CMV,[20,21] it does not necessarily cause a large amount of nephron damage and subsequent decrease in eGFR. Alternatively, infection caused by CMV may also be confined to the urinary bladder, causing cystitis without injuring the kidneys. Supporting data from a previous study showed that the most common form of post-transplantational CMV urinary infection in patients receiving HSCT was hemorrhagic cystitis.[22] Therefore, urinary CMV isolates may originate from the urinary bladder instead of the kidneys.

On the other hand, we found that the presence of CMV viremia during the post-transplantation period is closely associated with outcome in HSCT recipients with CMV viruria. CMV viremia has been linked to higher mortality in HSCT patients. Patients receiving HSCT often must take immunosuppressive medication. The immune responses of these patients to many infections, including CMV, are poor. Under those conditions, CMV infection or reactivation can easily occur and the virus can easily invade different organs if it reaches the blood stream. Unlike CMV detected in urine or bronchoalveolar lavage specimens, which indicate localized infection, CMV viremia may represent a systemic disease. Our findings suggest that blood CMV viral load testing may be required to determine the severity and predict the prognosis when CMV is detected in the urine.

Our study has some limitations. First, because this is a retrospective study, some important laboratory data, such as that of CMV viral loads in blood, were incomplete in some patients. Second, we searched the patient database from 2008 to 2014, but data were collected for only 28 patients. If urine CMV is detected by quantitative PCR or other more sensitive tests, a greater number of cases should be obtained.

In conclusion, for patients with hematological disease, antiviral therapy in asymptomatic CMV viuria is not associated with a clear clinical benefit whereas the presence of viremia indicates a poorer outcome. Further studies are needed to identify patients who may benefit from antiviral therapy in CMV viuria.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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