

Early abnormal liver enzyme levels may increase the prevalence of human cytomegalovirus antigenaemia after hematopoietic stem cell transplantation

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Abstract

Objective: Human cytomegalovirus (HCMV) infection is common after bone marrow transplantation (BMT), and it increases morbidity and mortality for transplant recipients. HCMV infection may cause hepatitis and elevate the liver enzymes aspartate transferase (AST) and alanine transferase (ALT). This study aimed to analyse the associations between liver enzyme levels and infection with HCMV antigenaemia after BMT.

Methods: Data from 30 patients after BMT were collected at different time points (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 months post-transplantation). The patients were divided into the HCMV antigenaemia-positive and HCMV antigenaemia-negative groups according to a peripheral blood pp65 antigen assay. Immunohistochemistry was used to identify HCMV pp65 antigen and conventional methods were used to detect liver enzyme levels.

Results: Twelve patients were pp65 antigenaemia-positive and 10 patients were positive in the first 3 months post-transplant. Liver enzyme levels were increased after positivity for HCMV antigenaemia ($p=0.034$ and $p=0.018$ for ALT and AST, respectively). One month before antigenaemia, AST levels were higher in the HCMV antigenaemia-positive group compared with the negative group ($p=0.006$).

Conclusion: HCMV antigenaemia mostly occurs in the early stage of post-BMT and early abnormal liver enzyme levels may increase the chance of HCMV antigenaemia after BMT.

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Keywords

Human cytomegalovirus, antigenaemia, bone marrow transplantation, liver enzymes

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Abbreviations

AST: aspartate transferase

ALT: alanine transferase

HCMV, human cytomegalovirus. * $P < 0.05$, comparison of the number of patients in the HCMV antigenaemia-positive and HCMV antigenaemia-negative groups at the same time point (Fisher's exact test, two-sided)

Introduction

Over the past half century, tremendous advances have been made in bone marrow transplantation (BMT). Despite these advances, infection is a severe complication in patients undergoing BMT.¹⁻⁴ Human cytomegalovirus (HCMV) is a common viral infection, which is a major factor in morbidity and mortality undergoing BMT.⁴⁻⁶ Rapid laboratory methods for the early detection of cytomegalovirus (CMV) are needed for preventing CMV disease in transplant recipients. Some sensitive and specific laboratory tests should be used to rapidly diagnose CMV infection after BMT. Detection of the HCMV antigen pp65 in peripheral blood mononuclear cells (PBLs) is preferred for screening for HCMV antigenaemia. This is because this method is more rapid and sensitive than culture and has a good positive predictive value compared with CMV-DNA by polymerase chain reaction.^{7,8} Wirgart et al.⁹ (1996) found that the best marker for monitoring kidney transplant patients might be the quantitative CMV pp65 antigen detection assay. In their study, positivity for antigen pp65 was used for screening for HCMV antigenaemia. HCMV infection may cause hepatitis and elevate the liver enzymes

aspartate transferase (AST) and alanine transferase (ALT).

Our study aimed to investigate the incidence of HCMV antigenaemia during 6 months post-BMT. Because HCMV hepatitis is associated with solid organ recipients and has little relation to BMT,^{10,11} we also investigated the associations between HCMV antigenaemia and ALT and AST levels at different time points post-BMT.

Methods

Patients

From January 2012 to January 2014, a total of 42 patients underwent allogeneic BMT in our hospital. Patients who were followed regularly for longer than 6 months after receiving transplantation in our hospital and provided written informed consent were enrolled in the study. The exclusion criteria consisted of clinical conditions that suggested a life expectancy shorter than 6 months, previous HBV, HCV, or HDV infections, and other liver diseases. A total of 30 patients completed the study. These 30 patients ranged in age from 14.5 to 48.0 years old, with a mean age of 30.50 ± 10.57 years. There were 13 men and 17 women. The characteristics of the 30 patients are shown in Table 1. There was no HCMV-seropositive donor in our series. Every hematopoietic stem cell donation and transplantation in our centre strictly followed the guidelines of the Ethics Committee of our hospital and the Declaration of Helsinki. Written informed consent was obtained from each of the participants prior to enrolment. The study was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang

Table 1. Characteristics of patients in the study.

	HCMV + (n = 12)	HCMV - (n = 18)	Statistical analysis
No.	12	18	
Age (years, mean \pm SD)	24.08 \pm 7.42	34.78 \pm 10.32	t = -3.089, P = 0.004*
Sex (M/F)	3/9	10/8	$\chi^2 = 2.738$, P = 0.098
Diagnosis (AML/CML/ALL)	6/2/4	5/7/6	$\chi^2 = 2.228$, P = 0.328
HCMV + before transplantation	0	0	

Abbreviations: M, male; F, female; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; HCMV, human cytomegalovirus. P < 0.05 (dependent-samples t test).

University (RES reference number: 2012-005).

Immunosuppressive therapy

Chemotherapy before transplantation consisted of busulfan (4 mg/kg/day from days -7 to -4) and cyclophosphamide (60 mg/kg/day from days -3 to -2). Prophylaxis for graft-versus-host disease consisted of mycophenolate mofetil (1.0 g/day for 3 months after BMT) and cyclophosphamide (3 mg/kg/day for 24 h by intravenous infusion, to maintain blood concentrations at 300–500 ng/ml until medicine could be taken orally at 6 mg/kg/day).

Antiviral treatment

All BMT recipients received ganciclovir intravenously at a standard dosage of 5 mg/kg/day until the day of transplantation. Antiviral treatment for HCMV infection was started in each BMT recipient according to a previously reported preemptive therapy regimen.^{12,13} Briefly, all patients received ganciclovir intravenously at a standard dosage of 5 mg/kg every 12 h for 14 days or until negative antigenaemia results were observed.

Methods of detection

From day +1 until day +90 after BMT, HCMV pp65 antigen monitoring was performed twice a month. From day +90 until 6 months after BMT, the pp65 antigen assay

was performed every month. At the same time, conventional methods were used to assay liver enzyme levels. Liver enzyme levels are usually less than 40 U/L for AST and less than 50 U/L for ALT. All of the patients were monitored twice weekly during the next 2 months after BMT, and then every month during the next 4 months post-6 months after BMT. A total of 296 blood samples were taken from the 30 patients. Immunohistochemistry was used to identify CMV pp65 antigen. The CMV antigen assay was performed as described previously, with minor modifications.^{11,14} A standard two-step immunohistochemical method was used to assay CMV antigen expression in PBLs. In brief, leukocytes were separated from EDTA-anticoagulated blood and spread on slides. Anti-CMV-PP65-Ag monoclonal antibody (DAKO, Denmark) and the EnvisionTM + System peroxidase (DAB) kit (DAKO) were used. The stained samples were analysed under an optical microscope with an image recording system (BH-2, OLYMPUS, Japan). Cells that were stained yellow or brown were positive and blue cells were negative. The results are shown as the number of positive cells per 50,000 leukocytes, as previously described.¹⁵

Diagnosis of HCMV antigenaemia

HCMV antigenaemia is defined by serological conversion to CMV in a patient who had been seronegative before transplantation. In our study, we used antigenaemia

as the gold standard for detection of CMV, as previously reported.¹⁶ The standard of antigenaemia is in accordance with a previous report.¹⁷ HCMV infection was diagnosed when more than one positive cell was observed among 50,000 leukocytes in the blood samples. The patients were divided into the infection group and the control group according to the peripheral blood pp65 antigen assay results.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 (Chicago, IL, USA). The chi-square test was performed for dichotomous variables. The dependent samples t test was used to compare differences in the median values of continuous variables. A P value of less than 0.05 was considered statistically significant.

Results

Twelve (40.00%) patients were classified into the HCMV antigenaemia-positive group, and 18 (60.00%) patients were classified into the HCMV antigenaemia-negative group. Ten of 12 patients were positive for HCMV antigenaemia during the first 3 months post-BMT. Two patients were positive for pp65 antigen in the first month post-BMT, four were positive in the second

month, four were positive in the third month, one was positive in the fifth month, and one was positive in the sixth month. In the HCMV antigenaemia-positive group, 10 patients' ALT levels were normal before antigenaemia and nine patients' AST levels were normal. In the HCMV antigenaemia-negative group, six patients' ALT levels were normal and eight patients' AST levels were normal. ALT levels between the two groups were significantly different ($P=0.0015$, Fisher's exact test, two-sided). However, AST levels were not significantly different between the groups ($P=0.323$, Fisher's exact test, two-sided). ALT and AST levels at different time points in the HCMV antigenaemia-positive group are shown in Table 2.

At the time point when HCMV antigenaemia was positive, ALT levels in six patients in the HCMV antigenaemia-positive group were elevated compared with two patients in HCMV antigenaemia-negative group. This difference of higher ALT levels in the HCMV antigenaemia-positive group than in the negative group was significant ($P=0.034$, Fisher's exact test, two-sided). AST levels were also significantly differently higher in the HCMV antigenaemia-positive group than in the HCMV antigenaemia-negative group ($P=0.018$, Fisher's exact test, two-sided). ALT and AST levels in the HCMV antigenaemia-positive group

Table 2. Comparison of ALT and AST levels at different time points in patients who were positive for HCMV antigenaemia.

Different time points in the HCMV antigenaemia-positive group	ALT		AST	
	Elevated	Normal	Elevated	Normal
One month before HCMV antigenaemia	0	10	1	9
At the time of positivity for HCMV antigenaemia	6	6	4	8
P	0.0015*		0.323	

AST: aspartate transferase; ALT: alanine transferase; HCMV, human cytomegalovirus. * $P < 0.05$, comparison of the number of patients in the HCMV antigenaemia-positive group among different time points (Fisher's exact test, two-sided).

Table 3. Comparison of ALT and AST levels between patients in the HCMV antigenaemia-positive and negative groups at the same time point.

	ALT		AST	
	Elevated	Normal	Elevated	Normal
HCMV antigenaemia-positive group	6	6	4	8
HCMV antigenaemia-negative group	2	16	0	18
P	0.034*		0.018*	

Abbreviations: AST: aspartate transferase; ALT: alanine transferase; HCMV, human cytomegalovirus. * $P < .05$, comparison of the number of patients in the HCMV antigenaemia-positive group and the HCMV antigenaemia-negative group (Fisher's exact test, two-sided).

and the HCMV antigenaemia-negative group are shown in Table 3.

Discussion

HCMV infects 70–100% of adults in populations worldwide.¹⁸ For BMT recipients, infection with HCMV is still a major problem regarding morbidity and mortality. Clear evidence shows that HCMV donor seropositive/recipient seronegative (D^+/R^-) BMT may increase the incidence of HCMV infection post-transplantation for recipients.^{19,20} There were no HCMV-seropositive donors in our series to reduce the incidence of transmission of HCMV infection by leukoreduced blood product.^{2,20}

In the present study, 83.33% (10/12) of patients were positive for HCMV antigenaemia during the first 3 months post-transplant. HCMV replication of most BMT recipients typically occurs after engraftment until approximately 100 days post-transplantation.¹ HCMV is a ubiquitous herpes virus that belongs to the β -herpes virus family. Similar to other herpes viruses, the cellular immune response plays a critical role in viral clearance after HCMV infection. Cell-mediated immunity for BMT recipients during the first 100 days post-transplantation is impaired because some infection may occur, particularly HCMV.^{1,20}

We found that HCMV infection was significantly associated with liver enzyme

levels. A previous study showed that ALT and AST values started to increase at the time of distinct CMV infection (day 12), suggesting an association between CMV infection and elevated liver enzyme levels.²¹

In our study, the number of patients whose ALT/AST levels were elevated at different time points in the HCMV antigenaemia-positive group and the HCMV antigenaemia-negative group was significantly different ($P < 0.05$). Specifically, ALT and AST levels at 1 month before HCMV antigenaemia were significantly elevated. Some studies have shown no significant differences between the infection and control groups regarding other factors that produce an increase in liver enzyme levels, such as age and the use of immunosuppressive agents after transplantation.^{22,23} Therefore, we suggest that there is a significant association between HCMV infection and an increase in liver enzyme levels (ALT and AST) before HCMV antigenaemia. HCMV induces activation of nuclear factor κB (NF- κB) after infecting fibroblasts and macrophage cells. NF- κB response elements are present in the enhancer region of the CMV major immediate early promoter, and activity of this promoter is strongly upregulated by NF- κB in transient transfection assays.²⁴ This activation of NF- κB may activate cytokines, which may lead to liver injury and increased ALT and AST levels.

We conclude that HCMV antigenaemia often occurs in the early stage of post-BMT, and that HCMV antigenaemia may be detected early by measures of liver function post-BMT. A limitation of our study was the relatively small number of patients, which might be associated with large interpersonal variation. Future larger-scale studies are required to verify the relationship between HCMV antigenaemia and early abnormal liver enzyme levels in patients who have BMT.

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