Management of Cytomegalovirus Infection after Living Donor Liver Transplantation

Running title: Cytomegalovirus infection after liver transplantation

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Original Paper, Liver section

Key words: cytomegalovirus, liver, transplantation

Abbreviation: Cytomegalovirus (CMV); living donor liver transplantation (LDLT); ganciclovir (GCV); valganciclovir (VGCV); mycophenolate mofetil (MMF).

Abstract

Background: Few studies on Cytomegalovirus (CMV) infection in adult-to-adult living donor liver transplantation (LDLT) have been reported. The aim of this study was to analyze the incidence, risk factors, and management of CMV infection after LDLT.

Methodology: Retrospective analysis was performed with 72 consecutive adult cases.

Results: CMV antigenemia was demonstrated in 31 (43.1%) patients and 9 patients (12.5%) manifested fever. Twelve patients were treated with intravenous ganciclovir (GCV) injection. There was improvement in 10 patients, while in one patient who had an adverse effect, foscanet was administered concomitant with CMV-IG, resulting in improvement, and the death of the another one from sepsis. Twelve patients were given oral valganciclovir (VGCV), and all showed improvement. ABO incompatible transplantation was associated with CMV infection after LDLT in both the univariate ($p=0.005$) and multivariate analyses ($p=0.04$). After discharge, 12 out of 63 patients (19%) suffered from CMV infection and all of them were taking steroid.

Conclusion: ABO incompatible transplantation was demonstrated as a risk factor for CMV infection during hospitalization. After discharge, immunosuppressive status seemed to be more essential as a predictor for CMV infection. Routine examination to detect CMV antigenemia is needed, especially in patients with potentially over-immunosuppressive conditions in out-patient clinics.

Introduction

Cytomegalovirus (CMV) is an ubiquitous herpes virus that infects 60 – 100% of humans, primarily in the first two decades of life (1-3). In most cases, after primary infection, CMV remains and individuals with normal immunity will not have any symptoms for the rest of their lives (3, 4). Around 40 to 50% of recipients undergoing liver transplantation suffer from CMV infection (4-6). Infection with CMV may occur asymptomatically (CMV infection) or as a symptomatic illness (CMV disease) (4, 7-9). In addition to its directly attributable effects, CMV is known as an immunomodulator virus that upregulates alloantigen, thereby increasing the risk of acute and chronic rejection and predisposing recipients to other opportunistic infections and Epstein-Barr virus-associated post transplant lymphoproliferative disorders (3, 8).

Although a marked decrease in the incidence and severity of CMV infection, and associated mortality after liver transplantation, has been achieved, CMV infection is one of the most common infectious complications after liver transplantation even now and is known to have adverse effects on transplant outcome (3, 4, 8). We herein review the present state of CMV infection in 72 consecutive adult living donor liver transplantation (LDLT) cases.

Methodology

Study population

From August 1997 to February 2008, 82 consecutive LDLTs were performed with 79 patients in our hospital. Nine patients who were younger than 15 years old and one patient whose medical record could not be located were excluded from this study. We assessed the remaining 72 cases until one year after LDLT. Out of 72 patients, 47 (65.3%) were male, and the age range was 16 to 68 years (mean: 51.5 years). The underlying liver diseases were as follows:

hepatitis C virus-related liver cirrhosis (LC) with or without hepatocellular carcinoma (HCC) (26 patients, 36.1%), hepatitis B virus-related LC with or without HCC (16 patients, 22.2%), fulminant hepatic failure (7 patients, 11.7%), primary biliary cirrhosis (6 patients, 8.3%), alcohol-induced LC (4 patients, 5.6%), graft failure (3 patients, 5.0%), congenital biliary atresia (2 patients, 3.3%), cryptogenic LC with or without HCC (2 patients, 3.3%), and non-alcoholic steatohepatitis, Budd-Chiari syndrome, primary sclerosing cholangitis, idiopathic portal hypertension, Caroli disease, and autoimmune hepatitis (1 patient each).

Immunosuppression after LDLT

Basically, the patients were administered a calcineurin inhibitor (tacrolimus or cyclosporine A) and corticosteroids as primary immunosuppressive therapy. Methylprednisolone was injected intravenously during surgery at a dose of 20 mg/kg and at a dose of 2 to one mg/kg/day tapered for 1 to 6 postoperative days followed by oral predonine at 0.3 mg/kg/day (7 to 28 days), 0.2 mg/kg/day (after 28days), and discontinued in 3 months to one year. If acute cellular rejection was proven, bolus injections of methylprednisolone were started. In addition to the above-mentioned therapy, the ABO-incompatible LDLT recipients underwent more intensive immunosuppressive therapy, in that they were given rituximab, a monoclonal chimeric human-murine anti-CD20 antibody, to deplete the B cells one week before surgery, and took apheresis and/or double-filtration plasmapheresis to reduce the anti-A or anti-B antibody titers perioperatively. To control the local intravascular coagulation in the graft, prostaglandin E1 for 3 weeks and methylprednisolone for 2 weeks were infused through a catheter that was put into the hepatic artery or portal vein. In addition, mycophenolate mofetil (MMF) was given as soon as possible after transplantation.

Surveillance in infection of CMV

Before LDLT, serum IgG against CMV was measured in both donors and recipients. After

LDLT, pp65 CMV antigens in peripheral blood leukocytes were determined once a week routinely until the recipients were discharged. After discharge, if the patients showed any clinical symptoms or findings, we carried out tests to detect whether the virus was present.
For the purpose of prophylaxis against virus infection, acyclovir or valacyclovir (after 2005) was given orally 3 months after LDLT.

Treatments

When CMV antigenemia was proven, ganciclovir (GCV) was given intravenously or valganciclovir (VGCV) orally as preemptive therapy or targeted therapy.

Risk factor analysis

The 18 variables were assessed in relation to CMV infection after LDLT (Table 1).

Statistical analyses

Univariate analysis was used to ascertain the relationships between each variable and CMV infection. The chi-square test or, for small numbers, Fisher's exact test was used for comparison of categorical data. Continuous variables were compared using the *t* test. A p value of <0.05 was considered statistically significant. For multivariate analysis, the factors identified as being associated with a p value $\leqq 0.10$ were entered into a stepwise logistic regression analysis to determine the independent risk factors for infections.

Results

CMV infection during hospitalization

After LDLT, 31 patients (43.1%) showed CMV antigenemia during their hospital stay. Of all the 31 patients, CMV disease occurred in 9 patients (12.5%), manifesting with fever. Among 22 asymptomatic patients, 4 were treated with GCV injection and 11 with VGCV and all of them were improved (Figure 1). One patient with no treatment died of multiple organ failure unrelated to CMV infection. Eight symptomatic patients were treated with GCV

injection and there was improvement in 6 patients. In the one patient, it was necessary to substitute foscanet and administer it concomitant with CMV-IG in place of GCV because of an adverse effect, namely, pancytopenia. Another patient died of Methicillin-Resistant Staphylococcus Aureus sepsis and multiple organ failure. One symptomatic patient was given VGCV with showing improvement.

The characteristics of the CMV-infected and non-infected patients are compared in Table 1. In the variables, ABO incompatible transplantation was found to be associated with CMV infection after LDLT in the univariate analysis (p=0.005). In the multivariate analysis, only ABO incompatible transplantation was found to be an independent predictor for CMV infection (odds ratio: 5.89, p=0.04) (Table 2).

CMV infection in outpatients

During the study period, 9 out of 72 patients died during their hospitalizations, and we therefore assessed the remaining 63 patients. CMV antigenemia was demonstrated in 12 patients (19.0%) after discharge (Table 3). Nine patients who did not show any symptoms and one suffering from fever survived with the treatment of GCV or VGCV. The remaining 2 patients showed CMV disease. A sixty-five year old female patient (#5), who had undergone LDLT for idiopathic portal hypertension 4 months before, complained of abdominal pain and diarrhea due to CMV infection. Although she was initially treated with intravenous GCV administration, she discontinued the use of the reagent because of pancytopenia due to hemophagocytosis. Foscanet was then given, resulting in death from perforation of the intestinal wall. Another patient, a 65 year-old woman who had received a transplant for hepatitis C virus-related LC with HCC 7 months before (#12), was admitted because of biliary stricture and cholangitis. She suffered from high fever and abdominal pain due to CMV

infection 11 months after the LDLT operation. Although GCV administration resulted in improvement of CMV antigenemia, she died of graft failure accompanied with biliary complication and Hepatitis C virus re-infection.

The 4 variables were assessed in relation to post-transplant CMV infection after discharge by univariate and multivariate analysis (Table 4). Among them, mycophenolate mofetil (MMF) administration and CMV infection episodes during hospitalization were demonstrated as risk factors by univariate analysis (p=0.03, 0.04, each). Both were possible predicting factors for CMV infection after discharge, but not to a statistically significant degree (p=0.06, 0.08, each).

Discussion

We initially did not have a defined policy of treating CMV antigenemia and no medication was given to the 7 patients who demonstrated CMV antigenemia without any related symptom. At the present, however, we start oral VGCG or intravenous GCV administration in all patients as soon as CMV antigenemia is demonstrated. The rationale for starting preemptive therapy has been reported to be the prevention of immunomodulation by CMV that could be associated with infections with other opportunistic pathogens, inhibition of further progression to CMV disease, and consequent saving of resource utilization or costs (10). VGCV is a valine ester prodrug of GCV, whose bioavailability is improved compared with GCV after oral administration, allowing the daily doses to be decreased (11), and this should allow patients to be given the medication without hospitalization with enough therapeutic efficacy (11). In our institute, VGCV has been given since 2005 as a preemptive therapy for CMV infection, and all 21 patients who showed CMV antigenemia improved on this drug.

Until now, various risk factors have been reported for CMV infection after liver transplantation (4, 8)(12). We analyzed 18 variables and only ABO incompatible transplantation was demonstrated as a risk factor for CMV infection in both univariate and multivariate analyses (Tables 1 and 2). The advantages and disadvantages of preemptive and universal prophylactic therapies with GCV or VGCV have been compared (2, 10, 13). We began giving acyclovir or valacyclovir to recipients after LDLT as prophylactic therapy, mainly for the purpose of preventing the Herpes Simplex virus and Varicella Zoster virus infection. Although prophylactic valacyclovir was demonstrated to reduce the incidence and delay the onset of CMV disease in renal transplantation recipients (14), little data are available on liver transplantation. While prophylactic therapy with GCV or VGCV was reported to reduce the rate of CMV infection or disease after solid organ transplantation, some reports demonstrated adverse effects of this strategy such as late-onset CMV infection and the emergence of drug-resistant CMV (8, 10). Because eight ABO incompatible transplant patients with CMV infection showed an identical onset time of infection after the transplant operation, the rate of CMV disease and CMV attributable mortality compared with ABO identical / compatible transplant patients (data not shown), starting preemptive therapy after CMV antigenemia detection is deemed to be reasonable option, as it is in the case of ABO identical / compatible transplant patients. In our series, we experienced one case of R (-) / D (+) transplantation. This situation is well known as a significant risk factor for CMV infection, and prophylactic therapy with GCV or VGCV was recommended in several reports (13, 15). We gave this recipient intravenous GCV immediately after the operation instead of oral valacyclovir, followed by oral VGCV for 5 months without CMV infection occurring.

In general, beyond 6 months, most transplant recipients do relatively well and suffer from the

same infections seen in the general population with minimum immunosuppressant given (17). Because, however, we have sometimes experienced CMV infection in LDLT patients after discharge, we followed patients for 1 year after their operation in this study. MMF was reported to be a risk factor for CMV disease in renal transplant recipients (18). In our study, MMF administration predisposed patients to CMV infection with marginal significance by multivariate analysis ($p=0.06$), and MMF is likely to be a potential risk factor. In our institute, MMF was given to patients, who were suspected of having acute cellular rejection with full doses of calcineurin inhibitor given, with ABO incompatible transplantation and to whom calcineurin inhibitor could not be given enough because of adverse effects, such as nephropathy. Additionally, all the patients were still taking steroids when they developed a CMV infection or disease after their discharge (Table 3).

In summary, preemptive therapy after LDLT with either intravenous GCV or oral VGCV seems to be a reasonable option in terms of therapeutic efficacy, even with an ABO incompatible graft. In the outpatient clinic, routine examinations as well as requested examinations for patients suspected of having CMV infection based on their symptoms, complaints, or findings are needed, especially under potentially over-immunosuppressive conditions. However, the interval of the examination and until when such examinations should be conducted remains to be elucidated.

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incidence and severity of primary cytomegalovirus infections and disease after renal transplantation. Nephrol Dial Transplant 2000; 15:711-714.

Figure legend

Figure 1. Outcomes of patients with post-transplant cytomegalovirus infection or disease.

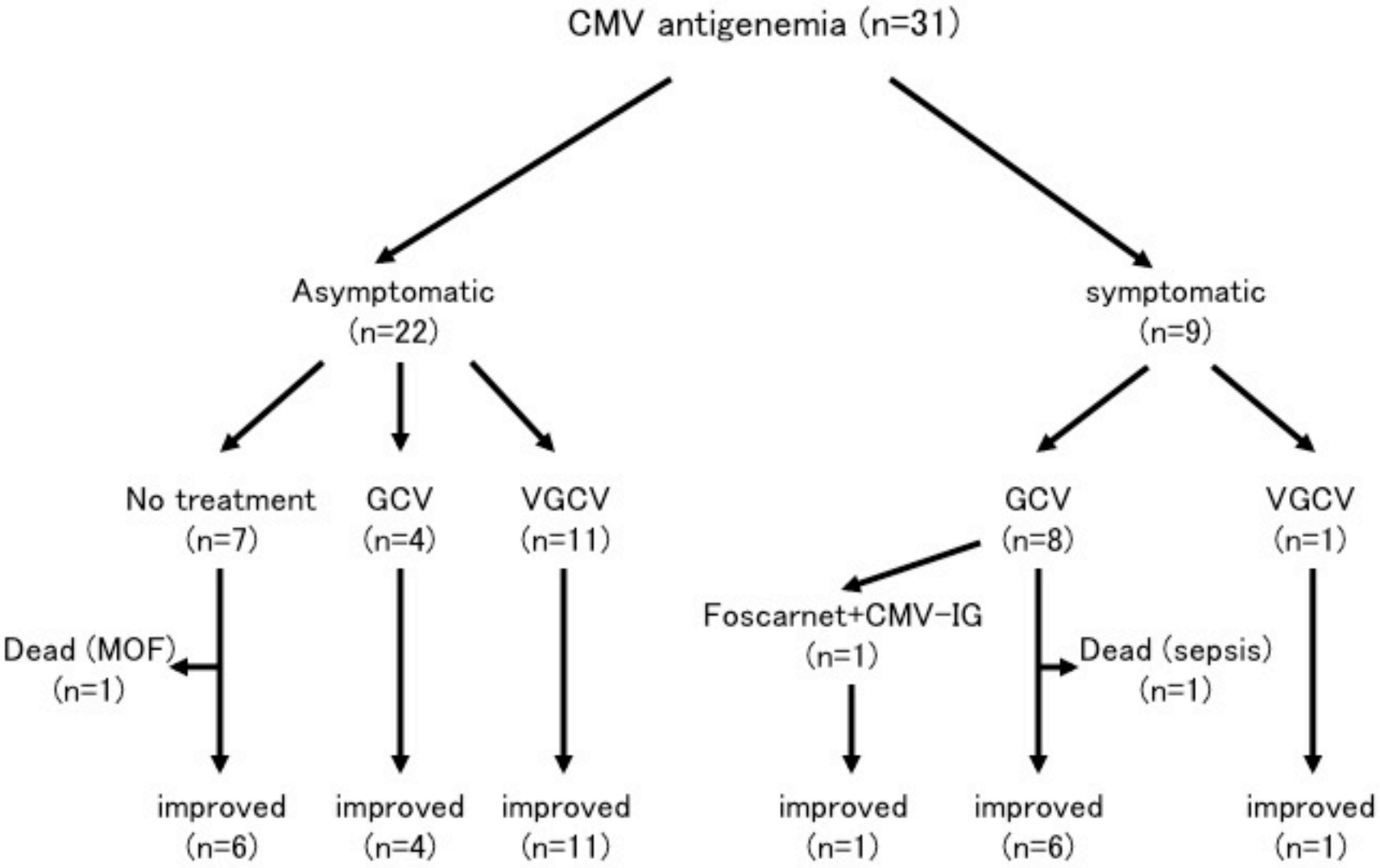
CMV antigenemia (n=31)
Asymptomatic
(n=22)
symptomatic
(n=9)
No treatment
(n=7)
GCV
(n=4)
VGCV
(n=11)
GCV
(n=8)
VGCV
(n=1)
Dead (MOF)
(n=1)
Foscarnet+CMV-IG
(n=1)
Dead (sepsis)
(n=1)
improved
(n=6)
improved
(n=4)
improved
(n=11)
improved
(n=1)
improved
(n=6)
improved
(n=1)

Table 1. Comparison of patients with and without CMV infection during hospitalization by univariate analysis

	CMV infected (n=31)	CMV non-infected (n=41)	P value
Age (year-old)	49.1	53.3	0.18
Sex (male)	20 (64.5%)	27 (65.9%)	0.91
Diabetes mellitus	6 (19.4%)	11 (26.8%)	0.50
Fulminant hepatic failure	4 (12.9%)	1 (2.4%)	0.10
HCV+/-HCC	11 (35.5%)	14 (34.1%)	0.91
MELD score	20.9	18.5	0.23
aGV/SLV (%)	46.3	50.4	0.21
Emergent LDLT	6 (19.4%)	2 (4.9%)	0.06
During of Surgery (minutes)	1,022	975	0.41
Amount of blood loss (ml)	13,109	14,320	0.78
Splenectomy	8 (25.8%)	8 (19.5%)	0.52
ABO-incompatible	9 (29.0%)	2 (4.9%)	0.005
Basiliximab injection	4 (12.9%)	5 (12.2%)	0.90
Mycophenolate mofetil administration	14 (45.2%)	11 (26.8%)	0.10
Acute rejection (pathologically proven)	12 (38.7%)	13 (31.7%)	0.54
Steroid bolus injection	6 (19.4%)	9 (22.0%)	0.88
Bacterial infection	15 (48.4%)	12 (29.3%)	0.10
Re-operation	11 (35.5%)	9 (22.0%)	0.20

NOTE: Data are numbers (%) of patients unless indicated otherwise.

Abbreviations: CMV, cytomegalovirus; MELD, the model for end-stage liver disease; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; aGV, actual graft volume; SLV, standard liver volume; LDLT, living donor liver transplantation.

Table 2. Multivariable analysis of risk factors for CMV infection after living donor liver transplantation recipients

Variable	OR (95% Confidence Interval)	p value
Fulminant hepatic failure	4.41 (0.15 – 131.33)	0.39
Emergent LDLT	2.92 (0.23 – 37.33)	0.41
ABO-incompatible	5.89 (1.05 – 33.06)	0.04
Bacterial infection	1.92 (0.62 – 5.89)	0.26
Mycophenolate mofetil intake	2.71 (0.88 – 8.32)	0.07

Abbreviations: CMV, cytomegalovirus; LDLT, living donor liver transplantation.

Table 3. The characteristics of the patients with CMV infection during out patient clinic

patient#	Age/Sex	Underlying liver disease	ABO incompatible	CMV antigenemia during hospitalization	Time after operation (months)	Immunosuppressant	Symptom	Treatment	Outcome
1	24/F	Biliary atresia	no	-	1	st+FK	none	VGCV	alive
2	16/M	FHF	no	+	4	st+CyA+MMF	none	VGCV	alive
3	55/F	HCV-LC/HCC	no	-	2/10	st+CyA+MMF	none	VGCV	alive
4	67/M	HCV-LC/HCC	no	+	2/4/6/8	st+FK+MMF →FK	none	VGCV	dead
5	65/F	Idiopathic portal hypertension	no	-	4	st+FK+MMF	Abdominal pain, diarrhea	GCV, foscanet	dead
6	25/M	Caroli disease	yes	+	8	st+FK+MMF	none	VGCV	alive
7	59/F	HCV-LC	no	+	6/9	st+FK+MMF	Fever/ none	VGCV	alive
8	33/F	HBV-LC/HCC	yes	+	5	st+FK+MMF	none	VGCV	alive
9	68/F	HCV-LC/HCC	yes	+	6	st+CyA+MMF	none	GCV	alive
10	52/M	HCV-LC/HCC	no	+	3	st+CyA	fever	GCV	alive

11	55/M	Cryptogenic LC	no	+	2	st+FK	none	GCV	alive
12	65/F	HCV-LC/HCC	no	-	8/11	st+CyA+MMF	none/fever, abdominal pain, diarrhea	GCV	dead

Abbreviations: FHF, fulminant hepatic failure; st, steroid; CyA, cyclosporine A; MMF, mycophenolate mofetil; VGCV, valganciclovir; HCV, hepatitis C virus; LC, liver cirrhosis; HCC, hepatocellular carcinoma; FK, tacrolimus; GCV, ganciclovir; HBV, hepatitis B virus.

Table 4. Comparison of patients with and without CMV infection during out patient clinic by univariate analysis

	CMV infected (n=12)	CMV non-infected (n=51)	p value
HCV-LC+/-HCC	6 (50.0%)	16 (31.4%)	0.19
ABO-incompatible	3 (25.0%)	5 (9.8%)	0.17
Mycophenolate mofetil administration	8 (66.7%)	16 (31.4%)	0.03
CMV infection episodes during hospitalization	8 (66.7%)	17 (33.3%)	0.04

NOTE: Data are numbers (%) of patients unless indicated otherwise.

Abbreviations: CMV, cytomegalovirus; HCV. Hepatitis C virus; LC, liver cirrhosis; HCC, hepatocellular carcinoma.