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Late cytomegalovirus disease following liver transplantation

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Abstract The widespread use of antiviral prophylaxis or preemptive therapy among orthotopic liver transplantation (OLT) recipients has reduced the occurrence of early cytomegalovirus (CMV) disease. Late disease is increasingly reported. Little is known about CMV disease occurring beyond the first year after transplantation. The aim of this study was to evaluate the occurrence of CMV disease two or more years after OLT and to determine its risk factors and clinical features. Eighty-one consecutive OLT recipients followed for 2 years or longer after transplantation were included in the study. Data were collected on demographic and clinical variables, clinical presentation, treatment, and outcome of late CMV disease. Late CMV disease occurred in 7/81 liver recipients (8.5%) at a mean time of 5.9 years after OLT (range: 3.5–9.3, median: 6.3 years). All seven patients were women, with a mean age of 47.7 years (range: 26–60, median: 59 years). There was no association between the development of late CMV disease and the occurrence of rejection episodes, treatment with corticosteroids, or the early use of antiviral prophylaxis. Clinical presentation included fever and disturbed liver functions in all patients, one patient had concurrent CMV pneumonitis and one CMV retinitis. Though all patients responded to ganciclovir, two had recurrent disease episodes and one patient died of secondary bacterial sepsis.

Late-onset CMV disease can occur several years after OLT. Although it manifests classic clinical features of early disease, it is not associated with traditional risk factors and its pathogenesis may differ from that of early disease.

Keywords Cytomegalovirus · Liver transplantation · Late infection

Introduction

Human cytomegalovirus (CMV) is a major pathogen in patients undergoing orthotopic liver transplantation (OLT) [1, 2, 3, 4]. Infection is common, with an incidence of 23–85%, while symptomatic disease develops in 15–40% [1, 3]. Clinical manifestations range from persistent fever with a mononucleosis-like syndrome to severe visceral disease such as interstitial pneumonitis, hepatitis, gastrointestinal involvement, and – rarely – retinitis [1, 5]. In addition to its direct effect, CMV has been implicated in immune modulation and associated with increased risk for bacterial and fungal infection as well as with allograft dysfunction [4, 5, 6, 7].

Several risk factors for CMV disease have been recognized, including the serological status of the donor and recipient, treatment with OKT3, high doses of methylprednisolone, and mycophenolate mofetil [1, 4, 8, 9, 10]. In the past, CMV disease was reported to occur mainly during the first 100 days after OLT [1, 2, 3, 4, 5]. The

widespread use of ganciclovir prophylaxis or preemptive therapy among OLT recipients has reduced the occurrence of early CMV disease [3, 4, 5, 11, 12]. Recently, however, the development of late-onset CMV disease (occurring beyond the first 100 days after OLT) has increasingly been reported [12, 13, 14, 15]. These reports suggest a possible shift in the natural history of CMV disease in OLT recipients. As yet, the clinical impact and risk factors of late CMV disease are poorly characterized, and surveillance data beyond the first year are scarce.

The aims of the current study were to assess the occurrence of late CMV disease 2 years and more after OLT and to determine its risk factors, clinical features, and outcome. Our findings show that late CMV disease could occur several years after OLT and should be considered in the differential diagnosis of patients presenting with fever and disturbed liver functions at any time after transplantation.

Patients and methods

Study patients

Eighty-one consecutive OLT recipients followed for 3 years or longer after transplantation were included in the study. The study patients were derived from a cohort of 200 OLT recipients treated at the Liver Unit, Hadassah Medical Center, Jerusalem, Israel. The charts, laboratory records, and histopathologic findings were reviewed retrospectively. Evaluated variables included: age and sex, underlying cause of OLT, recipient's CMV serostatus prior to OLT, donor CMV status (when available), use of CMV prophylaxis, rejection episodes and treatment, immunosuppressive regimen, CMV disease episodes and their time of onset after OLT, clinical characteristics, treatment, and outcome.

Forty-six patients were female and 35 male, the mean age being 49 years (range: 20–73 years). The indications for liver transplantation were hepatitis B virus (HBV) cirrhosis (19), hepatitis C virus (HCV) cirrhosis (18), cryptogenic cirrhosis (10), primary sclerosing cholangitis (PSC) (8), primary biliary cirrhosis (PBC) (8), autoimmune hepatitis (5), fulminant hepatitis (5), Budd-Chiari syndrome (2), hepatocellular carcinoma (2), and miscellaneous (4), including one patient each presenting with Wilson's disease, cystic fibrosis, hyperoxaluria, and hemangioendothelioma. The mean follow-up from the time of OLT was 6.3 years (range: 3–11 years).

Definitions

CMV infection

Diagnosis of systemic CMV infection was established by demonstration of positive pp65 antigenemia, defined by the presence of antigen-positive cells/2×10^5 WBCs, or isolation of CMV from buffy coat by culture on human foreskin fibroblast (HFF) cells, as previously described [16]. The presence of visceral or end-organ infection was dignosed by detection of viral antigens in the tissue and/or viral isolation from tissue or bronchoalveolar lavage by rapid shell vial assay or conventional cultures. The presence of CMV IgM and/or qualitative PCR was occasionally used to support the above findings.

CMV disease

Visceral and end-organ involvement were defined according to the recently updated criteria [17], the presence of compatible symptoms and signs accompanied by the isolation of CMV in blood or virologic and/or histologic detection in a biopsy specimen, in the absence of concurrent rejection or other infection.

CMV monitoring and prophylaxis following OLT

During the time of the study, patients were not routinely monitored for the presence of CMV infection beyond the first 3 months after transplantation, and laboratory investigation of CMV infection was employed only when infection or disease were clinically suspected. Most of the patients in our study group were transplanted before routine prophylaxis with ganciclovir became widely used. Of the seven patients with late CMV disease, five received CMV prophylaxis with acyclovir following OLT, while two did not receive prophylaxis. The regimen of acyclovir used for prophylaxis was 400 mg b.i.d. for 3 months. This dose is currently considered ineffective for CMV prophylaxis. As for the rest of the group, 44 were treated with acyclovir, 10 with ganciclovir, and 20 did not receive prophylaxis.

CMV treatment

Intravenous ganciclovir therapy (induction: 5 mg/kg b.i.d. for 3 weeks plus IVIG in the case of CMV pneumonitis) was initiated upon diagnosis of CMV disease, followed by maintenance treatment (5 mg/kg per day) in patients with CMV pneumonitis or retinitis.

Statistical analysis

The variables potentially associated with the development of late CMV disease 2 years or more after OLT were determined by univariate analysis. Comparisons between groups were performed by means of the χ^2-test, Fisher's exact test for categorical variables, or two-sample t-test for quantitative parameters.

Results

Demographic and clinical characteristics

Seven patients of the 81 OLT recipients evaluated had late CMV disease that occurred at a mean of 5.9 years after OLT (range: 3.5–9.3 years). All seven patients were women with a mean age of 47.7 years (range: 26–60 years). The underlying cause for OLT was HBV cirrhosis (2), HCV cirrhosis (1), autoimmune hepatitis (1), sarcoidosis (1), and PBC (2). All seven patients were CMV-seropositive prior to OLT.

One patient (patient 1, Table 1) had two successive episodes of CMV disease, and an additional patient (patient 6, Table 1) had three disease episodes. One patient (patient 5, Table 1) had an early episode of CMV disease that occurred 2 months after OLT and was treated with ganciclovir with complete recovery. The clinical presentation of late CMV disease was fever

Table 1 Demographic and clinical characteristics of the patients with late cytomegalovirus disease (*CMV* cytomegalovirus, *CyA* cyclosporin A, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *OLT* orthotopic liver transplantation, *PBC* primary biliary cirrhosis)

Factor	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)	32	59	61	62	34	60	26
Sex	F	F	F	F	F	F	F
Underlying cause for OLT	Sarcoidosis	HBV	PBC	PBC	Autoimmune hepatitis	HBV	HCV
Date of OLT	June 1996	Sept. 1996	April 1993	Jan. 1994	June 1994	May 1991	Nov 1993
Onset of CMV disease (years)	3.5	3.5	5.3	7	6.6	9.3	6.3
CMV disease episodes	2	1	1	1	1	3	1
Recipient CMV status	IgG+	IgG+	IgG+	IgG+	IgG+	IgG+	IgG+
CMV prophylaxis	Acyclovir	Acyclovir	Acyclovir	Acyclovir	–	–	Acyclovir
CMV prophylaxis duration (days)	180	100	100	100	–	–	365
Immuno suppression	CyA, 100 mg b.i.d.	Tacrolimus, 2 mg b.i.d.; prednisone, 5 mg/day	CyA, 75 mg b.i.d.; prednisone, 7.5 mg/day	CyA, 50 mg b.i.d.; prednisone, 5 mg/day	Tacrolimus, 2 mg b.i.d.; prednisone, 5 mg/day	Tacrolimus, 3 mg b.i.d.; prednisone, 5 mg/day	CyA, 50 mg b.i.d.; prednisone, 7.5 mg/day
Clinical presentation	Episode 1: Fever, hepatitis splenomegaly, pneumonitis Episode 2: Fever, retinitis	Fever, hepatitis	Fever, hepatitis	Fever, hepatitis	Fever, hepatitis	Fever, hepatitis, pneumonitis	Episode 1: Fever, hepatitis Episode 2: Fever, hepatitis Episode 3: Fever, hepatitis

and hepatitis in all patients. One patient had CMV pneumonitis and one developed CMV retinitis (Table 1). Six of the seven patients had rejection episodes after OLT that responded to methylprednisolone and did not require treatment with OKT3. There was no temporal association between rejection episodes and the development of late CMV disease in five of the six patients. In one patient, a rejection episode occurred 2 months prior to CMV infection. Five of the patients received CMV prophylaxis with acyclovir after OLT, while two did not receive prophylaxis. The mean time of prophylaxis was 169 days (range: 100–365 days). None of the patients developed early CMV infection while receiving CMV prophylaxis. At the time of development of CMV disease, six of seven were being treated with corticosteroids. The mean dose of prednisone was 5.8 mg/day. Three patients were treated with tacrolimus and four with cyclosporine. Mean trough serum levels were 7 ± 2 ng/ml (range: 3.3–12.9 ng/ml [normal range: 5–20 ng/ml]) and 130 ± 25 ng/ml (range: 65–265 ng/ml [normal range: 75–325 ng/ml]) for tacrolimus and cyclosporine, respectively, at the time of CMV disease diagnosis. No other known risk factors for CMV disease were found in our group of patients.

Treatment and outcome

All seven patients were treated with intravenous ganciclovir (induction with 5 mg/kg b.i.d. for a mean duration of 2 weeks, followed by maintenance treatment with 5 mg/kg per day or oral ganciclovir). Five patients made full recoveries. Although one patient had two recurrent episodes of CMV infection manifested as disturbed liver functions that occurred 12 and 18 months after the initial infection, she too made a complete recovery. One patient had CMV infection that manifested as fever with hepatitis, pneumonitis, and lymphadenopathy. She was treated with ganciclovir and made a full recovery. Six months after the first episode, the patient subsequently developed recurrent CMV infection that manifested as CMV retinitis. During her second hospitalization she developed bacterial sepsis and died.

Analysis of factors associated with the development of late CMV disease

We compared the group of patients that had late CMV disease with the rest of the cohort. Female gender was found to be in strong association with late CMV disease ($P = 0.029$ by Fisher's exact test). There was no association between the following parameters and late CMV disease: early CMV disease (occurring within 1 year of OLT), rejection episodes, treatment with either corticosteroids, tacrolimus, or cyclosporine, prophylactic treatment with acyclovir or ganciclovir, recipient CMV serology, or recipient age.

Discussion

The current shift in the epidemiology of CMV in transplant recipients towards late disease presents a challenge to the long-term success of liver transplantation. Little is known about late CMV disease occurring beyond the first year after transplantation. In the present study, we have shown the occurrence of late CMV disease, 3.5–9.3 years after OLT in seven patients, with an incidence of 8.5% among a cohort of 81 OLT recipients. All cases presented with fever and disturbed liver functions, while two patients had concurrent pneumonitis. Thus, the clinical manifestation of late CMV disease was "typical". Recently, late and atypical CMV disease in solid organ transplant recipients has been described [15]. The combined reports demonstrate the wide spectrum of manifestations associated with late CMV disease in this setting.

It is interesting to note that none of the well-characterized risk factors associated with early disease, such as age, rejection episodes, level of immunosuppression, and treatment with corticosteroids or OKT3, were associated with the development of late disease in the study patients. This is in contrast to the strong association demonstrated between early CMV disease, occurring within 190 days after OLT, and allograft rejection episodes [14]. The lack of association with traditional risk factors could suggest that the pathogenesis of late disease occurring years after transplantation may differ from that of late disease observed during the first year in the era of preventive therapy. We could not identify other potentially predisposing conditions associated with the so-called "cytokine burst" (i.e., tumor necrosis factor), such as intercurrent infection, myocardial infarction, or stress, in any of the patients with late CMV disease[18, 19].

The occurrence of late CMV disease in patients who were already seropositive at the time of transplantation could result from reactivation or from reinfection with another strain of CMV. In immunocompetent adults, CMV infection at a later age often results from close contact with young children [20, 21]. It is therefore noteworthy that all the patients with late CMV disease were women and female gender was statistically associated with late CMV disease. While none of the patients was working at a day care center, three of them were living with young children. Under these circumstances, reinfection could have potentially led to clinically significant disease, even in the absence of additional risk factors. Although previous reports in humans and in animal models have suggested that CMV activation can be induced by female reproductive hormones and – especially – estradiol [22, 23, 24], we do not think that this can explain the gender association.

Our study has several limitations. Due to its retrospective design, data on donor CMV status were unavailable. However, in view of the fact that all patients with late CMV disease were CMV-positive prior to OLT, we think that donor status is unlikely to play a major role in the pathogenesis of late disease in this group. Because our patients were transplanted at different centers and at a time when prophylaxis for CMV was not yet standard care, the prophylaxis regimen was variable, with some patients not receiving prophylaxis at all and the rest receiving acyclovir, which has poor protectivity against CMV infection. We believe, however, that our population, with a mean time after OLT of approximately 6 years (range: 3–11 years), reflects the general population of OLT recipients seen in many centers and that physicians caring for OLT patients are likely to encounter patients that received the prophylactic care common at the time of transplantation. Additionally, since the patients were not routinely monitored for CMV infection beyond the first 100 days after OLT, the study could have underestimated the incidence of late CMV infection. It remains to be seen whether standard prophylaxis or preemptive treatment with ganciclovir for a defined period of time will have an effect on late CMV disease.

Late CMV disease was associated with considerable morbidity. While the clinical response to ganciclovir therapy was generally good, two patients experienced recurrent disease, one of whom developed retinitis and subsequently died of secondary bacterial infection.

A recent report in stem cell transplant recipients suggested the policy of extended antigenemia surveillance during the first year in high-risk patients [25]. Due to the late and variable timing of disease development and the lack of characterized risk factors, such an approach does not appear feasible in the long-term follow-up of OLT recipients. Yet, in view of the late occurrence of disease some years after OLT, we suggest that clinical awareness should be maintained and that the diagnosis of CMV infection be considered in patients presenting with typical symptoms at any time after OLT. The high rate of recurrence suggests the need for closer monitoring of patients who experience late disease.

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