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REVIEW

CMV infection, diagnosis and antiviral strategies after liver transplantation

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Summary

Cytomegalovirus (CMV) is a significant pathogen complicating the post-transplant course of organ recipients. In liver transplant patients, the febrile clinical illness caused by CMV may be associated with end-organ disease, such as hepatitis or infection of the gastrointestinal tract. In addition to direct effects, CMV may have indirect effects including the risk of other infections or graft rejection. Recently, major advances in the management of CMV infection have been achieved through the development of new diagnostic techniques and antiviral strategies to prevent CMV disease. Quantitative nucleic acid testing to monitor viral load is now commonly used to diagnose and guide the treatment of CMV infections. The standardization of the testing, however, needs to be improved. There are two main strategies to prevent CMV disease after liver transplantation: prophylaxis and pre-emptive therapy. Both strategies are effective, but also have disadvantages. The disadvantages of prophylaxis include prolonged drug exposure, the development of resistance and, most of all, the development of delayed and late-onset CMV disease. On the other hand, the pre-emptive strategy is based on frequent laboratory monitoring of viral loads, and some patients may develop symptomatic infection before the diagnosis of CMV. This overview summarizes the current status of CMV in liver transplantation.

Introduction

Cytomegalovirus (CMV) is a major viral pathogen complicating organ transplantation. During the last two decades, major advances in the management of CMV infection of transplant patients have been achieved through the development of new diagnostic techniques and through the use of antivirals. Most transplant centers have protocols for the diagnosis and frequent monitoring of CMV, and strategies for treatment of the significant clinical infections and prophylactic and/or pre-emptive treatment have become common practice. In addition to clinical CMV disease and its risk factors, CMV-associated indirect effects, such as increased risk of acute or chronic allograft rejection, as well as other infections, have been recognized and addressed by these strategies.

Several guidelines for the management of CMV in transplant patient, diagnostic procedures and antiviral prevention of CMV by prophylaxis or pre-emptive therapies have been published within the last few years. The great number of review articles summarizing CMV-associated problems in stem cell and solid organ transplantation, clinical signs and symptoms, indirect effects, diagnosis, prevention and treatment protocols has provided a lot of information for the clinicians dealing with these patients. However, the severity and symptoms of CMV infection are not equal in all transplant patients, and there are significant organ-specific differences in this respect. The prevalence, clinical manifestations, direct and indirect effects associated with CMV depend on the transplanted organ and the patient population. This short overview is an attempt to summarize the current status of

CMV in organ transplantation, focusing mostly on adult liver transplant patients.

Epidemiology and pathogenesis

Cytomegalovirus is a widespread pathogen, causing an asymptomatic or mild mononucleosis-like primary infection, usually occurring in early childhood or in adolescence, with a seroprevalence in the adult population ranging from 30% to 100% according to age, geographical and socio-economical factors [1,2]. Although, no recent systematic analysis of CMV prevalence in healthy adults is available, based on previous observations and an analysis on stem cell transplant recipients, it is evident that even in European countries the overall seroprevalence varies widely being high, approximately 70–90%, in Southern and Nordic countries, whereas in the United Kingdom the seroprevalence of CMV is only 30–50% and in the Middle Europe 50–60% [1,3]. In Germany, the CMV-seroprevalence of liver recipients has been reported to be 65% [4]. However, 87% of the adult liver recipients were CMV-seropositive in our liver transplant center providing a representative sample of the Nordic population [5].Interestingly, it has also been reported that the proportion of CMV seropositive liver transplant recipients has decreased significantly over time, when the period 1989–1992 (86.4%) was compared with the period 2000–2003 (53.7%), whereas the donor CMV seropositivity remained unchanged [6]. The precise reason for this phenomenon was not clear and could not be explained, e.g., by the age of the recipients, but one possible suggested reason could have been pretransplant transfusion requirements [6].

Like other herpesviruses, CMV can establish latency.The virus may persist at specific sites in the host after primary infection, but without production of any detectable viral infection [7]. Sporadic reactivation events may occur, but they are generally well controlled by cell-mediated immunity, cytotoxic T cells and NK cells. The sites of latency are blood leukocytes, mainly mononuclear cells, but viral DNA has also been detected in the bone marrow hematopoietic progenitors, epithelial and endothelial cells. The latent virus can thus be easily transmitted by either the leukocytic or possibly even by the tissue cell population of the organ from the donor to the recipient. As the impaired cell-mediated immunity of transplant patients cannot control the virus, reactivation of the donor virus may occur especially in those CMV-seronegative recipients who have no immunity against CMV (D+/R−), but also in those recipients who are CMV-seropositive (R+) or those who simply undergo reactivation of their own latent virus. The risk of CMV infection is increased by immunosuppression with anti-thymocyte globulin and monoclonal T-lymphocyte antibodies, and other drugs, such as mycophenolate mofetil or combinations of steroids and tacrolimus, have also been reported to increase the risk of CMV [8,9]. Individual-specific immune responses may also affect the ability of liver recipients to control the virus [8,10].

Without prophylaxis, CMV infections occur in the majority of solid organ transplant patients, primarily during the first 3 months, when immunosuppression is most intense. CMV disease incidence ranges from 8% to 65% [11,12]. The risk factors for CMV disease in transplant recipients include CMV seropositive donor/CMV seronegative recipient (D+/R−) and the intensity of immunosuppressive therapy. Also, the type of transplantation has an effect on the occurrence of CMV disease: lung and heart-lung transplant recipients are at highest risk for CMV disease, liver or heart transplant recipients have an intermediate risk, and kidney transplant recipients are at the least risk [12]. Most of the CMV infections are caused by reactivation of latent virus of either recipient or donor origin.

Cytomegalovirus infection and disease are recognized as the predominant clinical problem among infections causing fever, hepatitis, neutropenia, thrombocytopenia, pneumonitis, gastrointestinal disease and retinitis. The definitions of CMV infection and disease in transplant recipients have been described [13]. 'CMV infection' is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen [13]. ° The minimum conditions for determination of 'CMV disease' are fever (>38 °C, for at least 2 days within a 4-day period), neutropenia or thrombocytopenia, and the detection of CMV in blood.In end-organ diseases, e.g., pneumonia, hepatitis, gastrointestinal disease, retinitis, nephritis, cystitis, myocarditis, pancreatitis or central nervous disease, detection of CMV requires to be performed from the organ in question. CMV pneumonia remains a life-threatening syndrome, which is usually complicated by other pathogens, such as fungal or bacterial co-pathogens [13]. In solid organ transplant patients, CMV can infect various organs such as lung, liver, intestines, kidney and heart, and it especially affects the transplanted organ [12]. In liver transplantation, a common end-organ disease is CMV hepatitis [11,12].

In addition to direct effects of CMV on the host, there are also indirect effects of the virus [14–16]. These include an association of CMV with acute graft rejection and chronic graft rejection, including accelerated transplant vasculopathy in heart transplant patients, chronic allograft nephropathy in kidney transplant recipients, bronchiolitis obliterans in lung recipients and vanishing bile duct syndrome (VBDS) after liver transplantation [16,17]. The development of other viral infections may also be increased, and CMV is thought to be a risk factor

for invasive fungal and bacterial infections in recipients [12,14].

Direct effects of CMV in liver transplantation

In liver transplant recipients, CMV infection is common and the overall incidence of CMV disease has been described as being up to 29% [12], and in the risk group of D+/R− it may be as high as 44–65% if no prophylaxis is given [8,18,19]. CMV hepatitis is a significant complication of CMV infection after liver transplantation with an incidence of 2–17% [4,5,12], and during the preganciclovir years, CMV hepatitis occurred even in 64% of the high risk (D+/R−) liver transplant patients [18]. The development of CMV hepatitis or intragraft CMV infection after liver transplantation does not necessarily correlate with high level of antigenemia in the blood, which means that CMV may infect the liver even in cases with relatively low viral loads [5]. However, CMV hepatitis seems to have no influence on the long-term outcome of patients, but biliary complications are found to be common [5,20]. CMV-associated biliary complications have been found to occur especially in the risk group of primary infections (D+/R−) with concomitant or preceding viremia [20]. CMV infection of the gastrointestinal tract, including esophagitis, gastritis, enteritis and colitis, is another common tissue-invasive complication in liver transplant recipients, often associated also with late onset CMV disease [8]. Gastrointestinal complications of CMV, such as gastroduodenal infection or colitis may also be found after liver transplantation without other signs of CMV disease or even without significant viremia [21–25].Other complications, such as pneumonitis, are less frequent in liver transplant patients, but occasional retinitis has been described after the discontinuation of CMV prophylaxis [26,27]. In general, however, attributable to modern diagnostic methods and effective antiviral strategies, the incidence and severity of CMV infection and end-organ disease are decreasing [4]. On the other hand, late onset CMV disease has become more common with the effective early prevention strategies [8].

Indirect effects of CMV in liver transplantation

In addition to clinical disease, awareness of CMV-associated indirect effects, such as increased risk of acute or chronic allograft rejection and the development of other infections, has increased [8,12–14,28,29]. CMV is thought to be a risk factor for invasive fungal and bacterial infections in recipients of liver transplants and associations between CMV and other viral infections have been reported [14,29–32]. CMV could also interact with other viruses and may accelerate hepatitis C virus pathogenesis [29,32]. On the other hand, human herpes virus 6 (HHV-6) and CMV infection and viral load were not associated with increased overall rates of HCV recurrence or HCV viral load after liver transplantation but may be associated with more severe forms of recurrence [33,34]. Concurrent beta-herpes virus activations are frequently found after transplantation and transactivations have been suggested [12,35,36].However, the activation of the other herpes viruses HHV-6, HHV-7 and Epstein–Barr virus has been reported together with CMV [12,33,35–37].

Cytomegalovirus is also suggested to be involved in liver rejection, and an association of developing VBDS and chronic rejection has been recorded [28,38–41]. CMV is an immunomodulatory virus, which is thought to be involved in alloresponse. CMV triggers the inflammation in the graft by upregulation of cytokines, MHC antigens and adhesion molecules, and induces various chemokines and growth factors [16,28]. In the liver transplant, CMV increases inflammation in the graft and the expression of class II molecules and certain adhesion molecules, such as ICAM-1, VCAM-1, and VAP-1, known to be important in leukocyte extravasation and T-cell activation [5,42–44]. In an experimental model, it was demonstrated that CMV increases the bile duct damage in conjunction with alloresponse [43].

It has previously been demonstrated that CMV may persist in the liver allograft for longer time. Persistent CMV-DNA could be demonstrated in hepatocytes, endothelial cells and also in bile duct epithelium several weeks after an acute infection, when no active virus was anymore found in the blood or any body fluids [39,40].In those series, persistence of CMV-DNA, and not acute CMV hepatitis, was associated with chronic rejection. Thus, successful antiviral treatment of CMV infection, does not exclude the persistence of the virus and the risk of chronic rejection, although most intrahepatic CMV infections do not affect the long-term outcome of the transplant [5,40].

Diagnosis of CMV infection

Since the late eighties, the semiquantitative CMV pp65 antigenemia assay has been used to diagnose CMV infection and assess the viral load in transplant patients. Immunostaining of the lower-matrix protein pp65 (UL83) in blood leukocytes provides a good clinical correlation of CMV disease with high numbers of positive cells [45,46]. Either immunoperoxidase or immunofluorescence techniques have been used. The most common modification of this method is a commercial one, based on immunofluorescence detection and counting of the CMV pp65 positive leukocytes. Although, there have been attempts to standardize the pp65 assay [45,47], various

in-house and commercial modifications of the method have rendered any effective comparison difficult. PCR techniques, which are less laborious and can be easily standardized and automated, are now used in most transplant centers [48–50]. Understanding of the correlation between viral load and clinical symptoms [51–53] has diminished the significance of qualitative methods and viral cultures. CMV-serology has practically no role in the post-transplant diagnostics, but is important in the pretransplant testing of the donor and recipient in order to detect the D+/R− risk patients.

Monitoring of viral load and clinical symptoms has diminished the use of qualitative nucleic acid detection methods in CMV diagnostics. However, certain qualitative molecular tests, such as detection of a late (pp67) CMV mRNA (NASBA), have still recently been in clinical use [46,50]. Nevertheless, experience suggests that this assay is less sensitive than DNA amplification and antigenemia tests [50,54,55].

The first commercial quantitative plasma DNA PCR assay, the Copas Amplicor Monitor, which has high sensitivity and specificity, demonstrated a good correlation with the clinical situation and with the kinetics of viral load [55–58]. Recently, new applications based on real time PCR technology have been developed. These fluorescence-based formats detect the accumulation of amplified product in real time and provide accurate quantification in a wide dynamic range. The real time quantification of viral genomes either by LightCycler-based PCR or TaqMan technologies are changing the practice of CMV diagnosis and monitoring [49,50,59–61]. It has been proposed to replace the pp65 antigenemia test by LightCycler or TaqMan technology, which has high sensitivity, cost-effectiveness and simplicity [59,61]. Many new PCR procedures have been applied for whole blood [49,59,61], but the use of other blood compartments is also possible [62,63]. The new quantitative methods are commercially available, but many laboratories use in-house PCR tests satisfactorily [64]. However, the optimal cut-off levels may vary between the methods [65,66]. In addition, not only the viral load, but also the load kinetics, faster rate of increase in viral load and replication rate of the virus, indicate a high risk of CMV disease or recurrent infection [53,67].

Guidelines for the management and diagnosis of CMV infection have been published by various specialist groups [68–71]. In the diagnosis of CMV and monitoring of viral load, quantitative PCR is mostly used, though the antigenemia assay is also accepted. The CMV DNA levels in whole blood are higher than in plasma, and whole blood is the sample of choice, although assays based on plasma specimens also are acceptable. Commercial methods are better controlled and preferable, but many real-time in-house assays are more sensitive, have broader linear range and faster turnaround times. Currently, the tests used vary widely with respect to their sensitivity, sample type, amplification targets and calibration standards. There is a need for an international standard to be set by an independent quality control organization [66,72]. However, the diagnostic procedures and the management of patients are still strongly influenced by the availability of modern nucleic acid technology and the local practice of transplant physicians.

Diagnosis of tissue-invasive CMV infection after liver transplantation

Cytomegalovirus infection of the liver transplant is characterized by graft dysfunction, i.e., elevation of serum transaminases, together with a positive CMV finding from the blood. The diagnosis must, however, be based on liver biopsy [13,73,74]. The histologic alterations associated with intrahepatic CMV infection may cause differential diagnostic problems, as the characteristic CMV inclusions are rarely recorded, and the other changes are rather nonspecific. In addition to the CMV-specific antigens in the graft, microabscesses, although not specific for CMV [5,74], are the most frequent findings. Additional findings, such as portal lymphocytic infiltration, some degree of endotheliitis and cholestasis may, however, lead to the misinterpretation of mild acute rejection. The demonstration of CMV antigens by immunohistochemistry or CMV-DNA by *in situ* hybridization in the biopsy specimens is necessary for the diagnosis of intrahepatic CMV infection [5,13,73,74]. To confirm the diagnosis of CMV-associated biliary complications, the viral antigens or DNA should, if possible, be demonstrated from the bile duct specimens [20]. Gastrointestinal CMV infection should, also be demonstrated from the endoscopic biopsy specimens by these methods [24,75,76]. PCR or culture methods alone are not suitable for the definitive diagnosis of a tissue-invasive infection, because they cannot localize the virus and the positive finding might be attributable to blood background and viremia [13]. However, positive culture findings or high viral loads in bronchoalveolar lavage (BAL) tissue-invasive infection give some evidence of pulmonary infection, though CMV antigen detection in the cellular components of BAL or in a transbronchial biopsy may be even more diagnostic [13,77].

There are two commonly used methods to detect CMV antigens in the tissue specimens. It is possible to demonstrate CMV antigens in frozen sections of liver biopsy specimen by using a monoclonal antibody against CMVpp65 matrix protein and immunoperoxidase staining [5]. This is a sensitive method, but does not work for paraffin-embedded formalin-fixed specimens. Thus, many histologists use an alternative immunostaining method

and a monoclonal antibody against CMVp52 delayed early antigens. This method is commercial, works on formalin fixed specimens and is easy to automate. The later method is especially good in the detection of viral inclusions, but the pp65 antigen detection might be more sensitive [24]. *In situ* hybridization to detect viral DNA in tissue specimens is a time-consuming and laborious method, but may also be used also for both frozen sections or formalin-fixed material [5,13,24,39,40]. Persistent CMV-DNA may be found in some cases even in the biopsies which are negative for CMV antigens [39,40].

Antiviral strategies and management of liver transplant patients

There are two main strategies to prevent CMV disease after liver transplantation; prophylaxis and pre-emptive therapy. CMV prophylaxis is based on the administration of antiviral drugs such as ganciclovir or valganciclovir to patients who are at risk of developing CMV disease. The efficiency of CMV prophylaxis initially after liver transplantation [19] and later in overall solid organ transplantation [78], has been proven to reduce the incidence of CMV disease. This strategy is recommended for all D+/R− recipients [69] to prevent primary infections, but is nowadays often used also for other patients [72]. Prophylaxis is mostly given for 3 months after transplantation [69,72]. The advantages of universal prophylaxis include the easy management, reduced incidence of CMV at the early stage after transplantation, and possibly less indirect effects of CMV infection, such as other opportunistic infections and rejection [79–81]. Pre-emptive therapy is based on the detection of CMV reactivation before the onset of clinical symptoms. This strategy is recommended mainly for moderate or low risk patients, such as R+ recipients [69]. However, some have successfully used pre-emptive strategy also for high risk patients (D+/R−) [82]. Early administration of antivirals, ganciclovir or valganciclovir, based on monitoring of viral load mostly by sensitive quantitative nucleic acid tests like quantitative CMV-PCR methods, may prevent the development of CMV disease. The advantages of pre-emptive therapy include reduced drug expose and toxicity, as well as less resistance problems and no late-onset CMV disease [82,83]. Meta-analyses of placebo-controlled trials have shown that prophylaxis might be a superior strategy in preventing CMV and the indirect effects of the infection [84–86], but also universal prophylaxis and pre-emptive therapy have been found equally effective in reducing the incidence of CMV disease [87].

Both strategies also have their disadvantages [8,88,89]. The disadvantages of universal prophylaxis include prolonged drug expose, the development of resistance and, most of all, the development of delayed and late-onset CMV disease [8,90–93]. Prophylaxis does not prevent the development of primary CMV infection; it only delays the onset of viral replication, and primary CMV infections are relatively common after the cessation of prophylaxis. The controlled prospective PV16000 trial demonstrated that 3 months prophylaxis either with valganciclovir or oral ganciclovir in solid organ transplant population is effective, but the overall 6-month rates of CMV disease were 12% and 15% respectively [79]. However, in the subgroup of liver transplant recipients CMV disease occurred in the valganciclovir group more frequently (19%) than in the oral ganciclovir group (12%) and there was also higher incidence of tissue-invasive CMV disease respectively. Because of these results, valganciclovir was approved by the US FDA for prophylaxis against CMV disease in other organ recipients, but not liver. In a recent study, the frequency of delayed-onset of CMV-disease in the D+/R− liver transplant recipients is reported to be relatively high with 3 months prophylaxis with either oral ganciclovir (22%) or valganciclovir (28%) [94]. The 2-year overall incidence of CMV disease after prophylaxis was 29%, but majority (90%) of the cases occurred within 100 days after prophylaxis was stopped. The median time of the diagnosis of CMV disease was 153 days after liver transplantation or 55 days (range 25–651 days) after cessation of antiviral prophylaxis. In addition to CMV syndrome, tissue-invasive CMV disease, mostly with gastrointestinal involvement, was recorded in more than half of those patients with delayed onset of primary CMV infection [94]. However, the rate of allograft loss or mortality did not differ from that of the patients who did not develop CMV disease [94]. In another series of liver transplant risk patients, CMV disease occurred in 26% after 3–6 months valganciclovir prophylaxis [95]. The delayed onset CMV disease is characterized by fever, bone marrow suppression and tissue-invasive infection, often affecting the gastrointestinal tract [8,91,94]. A systematic review in the literature has recently demonstrated the significant difference in the incidence of late-onset CMV disease as a disadvantage associated with the prophylaxis strategy versus pre-emptive therapy, especially in D+/R− solid organ transplant patients (17.5% vs. 8.9%) [96]. In liver transplantation, the overall incidence of delayed onset primary CMV disease has been reported to range from 16–47% in patients with prophylaxis [8]. Whether prolongation of prophylaxis to 6 or even to12 months would prevent late-onset CMV disease is still under discussion [8,89].

On the other hand, pre-emptive therapy is a more complicated procedure to organize and is highly dependent on frequent laboratory monitoring and the rapid logistics of the specimens. If the patients are monitored

weekly or once in 2 weeks, this might result in the onset of clinical illness prior to laboratory detection of CMV. This could particularly be the case in D+/R− patients, in which the replication kinetics of the virus might be very rapid [53]. The laboratory monitoring strategy, standardization of methods, agreement of cutoff levels of viral loads and optimal pre-emptive drug regimens are still far from international consensus, although various specialist groups have published their guidelines and recommendations [68–71]. Until these problems are resolved, most transplant centers either run their own local strategies, or just simply use prophylaxis. In a recent survey of CMV prevention strategies of 110 liver transplant centers (106 US, 4 Canada), it was clear that prophylaxis was preferred over pre-emptive therapy not only in the D+/R− risk group (77%), but also in D−/R+ (60%) and D+/R+ (54%) patients [72]. Most centers used valganciclovir for both prophylaxis and for pre-emptive therapy. In liver transplantation, valganciclovir has in fact, though not approved by US FDA, replaced high-dose aciclovir and oral ganciclovir, which were previously used for prophylaxis. The quantitative CMV-PCR test was found to be the primary diagnostic procedure measuring the viral loads and to determine to which degree of DNA elevation in the blood to predict the development of CMV disease [72]. In Europe, the pre-emptive strategy is probably more common than in the USA [46], and is mainly used for R+ recipients, and prophylaxis given to the high risk D+/R− patients, according to previous recommendation [69]. However, both strategies are effective and have decreased the overall incidence of CMV disease [96].

The antiviral treatment of CMV disease has until recently been mostly managed with intravenous ganciclovir therapy. However, recent results of the series on the safe use of valganciclovir also in the treatment of symptomatic infection are very promising [97,98]. Other antivirals, which are effective in the treatment of CMV infection are foscarnet and cidofovir. However, their role in liver transplantation has remained minor, partly because of their toxicity, and they may be used in case of ganciclovir resistance. Unfortunately, cross-resistance is common, especially in the cases of DNA-polymerase UL54 mutations. Hyperimmune globulin is occasionally administered combined with the specific antiviral treatment in a few cases of life-threatening CMV-pneumonia, but not usually in liver transplant patients. There are also some new drugs under investigation, but their efficiency in preventing CMV will be seen in the future.

Conclusions

Although, the new therapeutic procedures and the use of modern diagnostic methods have reduced the incidence of severe infections, CMV still remains a significant pathogen in liver transplantation. There are two main strategies to prevent CMV disease after liver transplantation; prophylaxis and pre-emptive therapy. Both strategies are effective, but also have disadvantages. The direct and indirect effects may be reduced by prophylaxis with antivirals, to-day mostly with valganciclovir, though late primary infections may complicate the post-transplant course of the patients. Also a great number of CMV-seropositive recipients, who would never develop CMV reactivation, are drug-exposed with prophylaxis. On the other hand, pre-emptive strategy is based on the frequent laboratory monitoring of viral loads, and some patients may develop a symptomatic infection before the diagnosis of CMV viremia. Ganciclovir resistance, late-onset infection and the choice of optimal prevention strategy including standardized diagnostic procedures, remain the main CMV-associated challenges of this decade.

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