REVIEW ARTICLE

Human cytomegalovirus infections in solid organ transplant recipients: An updated review.

Mohd Fahmi Mastuki[1,2], Niazlin Mohd Taib[2], Siti Norbaya Masri[2], Mohd Azmi Mohd Lila[3], Siti Nazrina Camalxaman[1]

[1]Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA Cawangan Selangor Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia; [2]Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia, [3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

[*]**Corresponding Author**

Mohd Fahmi Mastuki
Email:
mohdfahmi@uitm.edu.my

Abstract:

Human cytomegalovirus (HCMV) is an important cause of morbidity and mortality in immunocompromised individuals such as transplant recipients and paediatric patients. The approach to defining clinical syndromes related to the virus, diagnostic techniques, treatment, and prevention strategies have developed over time. This article provides an updated overview of the epidemiology, clinical outcomes, diagnosis, treatment, and prevention of HCMV disease in solid organ transplant (SOT) recipients.

Keywords: Cytomegalovirus, diagnosis, epidemiology, immunocompromised, transplantation.

1. INTRODUCTION

Human cytomegalovirus (HCMV) is a worldwide prevalent herpesvirus that commonly infects humans (Razonable & Humar, 2019; Staras et al., 2006). It becomes latent following primary infection but reactivates recurrently and causes disease in solid organ transplant (SOT) recipients in the setting of the immunocompromised (Kotton et al., 2018). After transplantation, active HCMV infection and disease are associated with an increased risk of graft failure and fatality; thus, HCMV prevention approaches are generally applied in such group. Preventative therapy reduces reactivation in the setting of latent infection in the transplant recipient and acquisition of acute infection in HCMV-seronegative recipients of seropositive donors. However, HCMV disease may still manifest despite preventative treatments, particularly when they have not been dosed appropriately It also occurs after discontinuation of the treatment (Kotton et al.,2018; de la Torre, et al., 2011).

2. REVIEW METHODS

A methodical literature search was conducted on human cytomegalovirus and solid organ transplantation from 1990 to 2022. Boolean operator, truncation and phrase searching were the search techniques employed to find articles discussing on the virus epidemiology, patient management and viral-host factors associated with HCMV disease in the database of Sciences Direct, Web of Science, MEDLINE, PubMed, EBSCO Host Journals and Google Scholar.

3. HCMV EPIDEMIOLOGY, MANAGEMENTS AND ASSOCIATED FACTORS OF HCMV DISEASE.

3.1. Virology

HCMV is a β-herpesvirus that shares many of the characteristics of other herpesviruses. It has a huge, double-stranded DNA genome with a viral capsid and envelope, and core of proteins preserved within all the herpesviruses (Fishman, 2013). The pathogenesis of HCMV in otherwise healthy individuals is guided by viral transcripts that interfere with viral antigen presentation on the cell surface and lead to lytic viral replication. Symptoms that occur with viral replication are generally associated with the immune response to the infection rather than direct viral destruction; as a result, symptoms might be reduced in immunocompromised hosts with an inadequate immune response (Fishman, 2013). Most healthy hosts are asymptomatic with HCMV infection however shed virus in saliva and other mucous membranes, leading to transmission in between individuals (Fishman, 2013).

HCMV infects epithelial cells, lymphocytes, and monocytes and eventually establishes latency within these cells along with macrophages with a balance of viral immune evasion mechanisms and host immune control. The varied interactions of HCMV with the body immune system and the balance in between diseases make the viral replication versus graft rejection present hurdles for efficient management of infection in immunosuppressed SOT recipients (Ljungman, et al., 2017).

3.2. Epidemiology and risk factors

Seroprevalence rates of HCMV in the overall population have been reported to vary from 30% to 97%. Therefore, it is likely that many children will certainly acquire an HCMV infection during their lifetime (Bedel, 2012). HCMV is ubiquitous, and transmission occurs horizontally through person-to-person contact with virus-containing secretions from contaminated individuals (saliva, urine, genital secretions), vertically from mother to baby (in utero, during delivery, or postnatally via breastfeeding), through transfusions of blood products from infected donors, and with SOT from infected donors (Kimberlin, 2018).

In the setting of SOT, the primary risk factor for HCMV infection or disease is the HCMV serostatus of the donor/recipient pair. Recipients who are HCMV seronegative before transplantation and obtain an organ from a seropositive donor ([D]-positive/ recipient [R]-negative: D+/R-) are at high risk of primary HCMV infection at the time of transplantation that subsequently progresses to HCMV disease (Martin & Danziger, 2011). Children are more likely to be HCMV seronegative at the time of transplantation than adults and are at higher risk of acquiring HCMV from their organ donor, particularly if the donor is an older child or adult. Prior to the routine use of antiviral prophylaxis, the rate of symptomatic HCMV infection amongst liver transplant recipients had been reported to be 20% to 60% within the first 30 to 90 days after SOT (Bedel, 2012).

Immediate initiation of immunosuppression could enhance the risk of HCMV disease amongst SOT recipients with donor and recipient seropositivity mismatch. SOT recipients who are formerly HCMV infected before transplant (R1) are at risk of developing HCMV reactivation after initiation of immunosuppression after SOT. The risk of HCMV disease among SOT recipients also varies depending on the type of transplanted organ and variation in immunosuppressive methods to prevent graft failure. Lung and small intestine transplant recipients have a higher risk of developing HCMV disease, even in the circumstance of recipient seropositivity before transplant, whereas liver, heart, and kidney recipients have a lower risk of reactivation (Martin & Danziger, 2011).

Table 1. Risk assessment of SOT recipients based on donor and recipient serostatus.

Organ	Serostatus		Risk Assessment
	Donor HCMV	Recipient HCMV	
Kidney, liver, heart	Negative	Negative	Low
	Positive or negative	Positive	Intermediate
	Positive	Negative	High
Lung, intestine	Negative	Negative	Low
	Positive or negative	Positive	High
	Positive	Negative	High

3.3. Clinical manifestations of HCMV

Primary HCMV infection in immunocompetent persons is usually asymptomatic but may appear as a self-limited febrile illness or mononucleosis-like syndrome prior to developing life-long latency (Kimberlin, 2018). Among immunocompromised individuals, clinical manifestations might vary significantly from asymptomatic replication to HCMV-associated end-organ disease. Updated definitions to define the numerous groups of HCMV infection and condition have recently been published to standardize reporting of HCMV-associated outcomes among transplant recipients, considering advanced diagnostic testing techniques (Ljungman, et al., 2017). Additional definitions are used use to define HCMV features in patients with hematologic malignancies or those undertaking Hematopoietic stem cell transplantation (HSCT). These definitions are recommended (Razonable & Humar, (2019):

- HCMV infection: presence of HCMV replication in tissue, blood, or other bodily fluids irrespective of symptomatology (this term is different, and should be distinguished, from latent HCMV). HCMV replication is detected by (i) nucleic acid testing (NAT), (ii) antigen testing, and (iii) viral culture. Depending on the method used, HCMV replication in the blood can be termed as HCMV DNAemia or RNAemia (NAT), HCMV antigenemia (antigen testing), and HCMV viremia (culture) (Razonable & Humar, (2013).

- HCMV disease: HCMV infection that is accompanied by clinical signs and symptoms. HCMV disease is characterized into (i) HCMV syndrome, which typically manifests as fever, malaise, atypical lymphocytosis, leukopenia or neutropenia, thrombocyte-penia, and elevated hepatic transaminases, and (ii) end‐organ HCMV disease (e.g., gastrointestinal disease, pneumonitis, hepatitis, nephritis, myocarditis, pancreatitis, encephalitis, retinitis, others). HCMV tends to enter the transplanted allograft (Lisboa et. al., 2011); hence, HCMV more frequently causes nephritis in kidney recipients, hepatitis in liver recipients, or pneumonitis in lung recipients. The consensus definitions for these HCMV diseases were recently published (Razonable & Humar, 2013).

- Asymptomatic HCMV infection: HCMV replication without clinical signs and symptoms of disease (Bedel et al., 2012). HCMV has some indirect consequences leading to part from its capability to regulate the body's immune system. HCMV has been associated with increased risks of other infectious complications such as bacteremia (Ljungman, et al., 2017; Ljungman et al, 2008), invasive fungal diseases and Epstein‐Barr virus‐mediated post‐transplant lymphoproliferative disorders (Razonable & Humar, 2019). HCMV infection is associated with acute rejection and chronic allograft injury, consisting of chronic allograft nephropathy (Helantera et al., 2011; Arthus et al., 2008; Kliem et al., 2008; Witszke et al, 2012), bronchiolitis obliterans (Zamora 2002), and coronary vasculopathy (Potena & Valantine, 2007; Valentine, 2004). A significant association between HCMV infection and a reduction in patient's survival is well explained in numerous research studies (Arthus et al., 2008; Beam et al, 2016; Arthus et al., 2010).

Implementation of prophylaxis therapy with antiviral drugs during this onset after SOT has shifted the timeline for HCMV reactivation and disease to stage after the treatment has ceased. Immunocompromised children who acquire primary HCMV infection via community exposures are at threat for extended HCMV replication and disease, especially SOT recipients who remain to take lifelong immunosuppression, compared with immunocompetent children in the same setting. Primary HCMV infection or reactivation in the setting of immune disorder as the effect of immunosuppression might likewise precipitate the indirect effects of HCMV infection, consisting of organ rejection, Graft versus host disease (GvHD), and disease due to opportunistic pathogens.

3.4. Diagnosis

The diagnosis of HCMV disease in solid organ transplant recipients depends on the clinical history, presentation, and laboratory findings. It is important to interpret the laboratory findings appropriately as the virus, HCMV genetic materials and HCMV antigen can be identified in both patients with and without active disease. Appropriate diagnostic testing is fundamental for monitoring HCMV infection and disease in immunocompromised patients. Several diagnostic approaches are available such as molecular testing, pp65 antigenemia, serology, histopathology, and resistance testing (Table 2).

Quantitative PCR and Antigenemia Assays

Many studies have indicated the clinical value of PCR testing in diagnosis and monitoring HCMV disease after solid organ transplantation (Razonable & Hayden, 2013; Aitken et al., 1999; Humar et al., 1999; Sia et al., 2000a; Sia et al., 2000b). Detecting viral load in the initial phase of infection and the level of rise in viral load may aid in identifying patients at risk for HCMV disease. The assay has shown a higher viral load in patients with active HCMV disease compared with those with asymptomatic infection (Cope et al. 1997; Ferreira et al, 1999; Roberts et al., 1998; Weinberg et al.,2000; Hassan et al., 1999). These approaches have been applied routinely on solid organ transplant recipients to detect active HCMV disease, screen patients for the use of pre-emptive antiviral therapy, and monitor responses to antiviral therapy.

Although HCMV pp65 antigenemia test is also available for HCMV detection, quantitative PCR assays provide several benefits over the assay, including better assay standardization, increased sample stability, smaller sample quantity is required, and the capability to test patients with leukopenia. For these reasons, quantitative PCR assays are more widely used than the antigenemia assay; thus, it is chosen for diagnosing and determining of immunocompromised patients with HCMV infection and disease (Kotton et al., 2018; Eguchi et al., 2017).

Plasma or whole-blood specimens are commonly used for HCMV viral load assay. Viral load is usually higher and detected more frequently in whole blood as compared to plasma as both cell-free and intracellular viruses can be detected. Viral load levels in most patients are found to be 1 log (10-fold) higher in whole blood compared with plasma (Lisboa et al., 2011). However low levels of HCMV DNA in whole blood or plasma do not constantly correlate with active HCMV disease despite assays with whole blood being more sensitive than plasma (Razonable et al., 2002). For storage, HCMV DNA is stable in both type of samples for up to five days when kept at 4° C (Caliendo et al., 2000; Yen et al., 2001) and three to four days while at room temperature (Yen et al., 2001; Schäfer et al, 1997). One study showed that the plasma DNA is steady for 14 days at 4° C once it is separated from whole blood (Abdul-Ali, 2011). The best anticoagulant for quantitative HCMV PCR assays is ethylenediaminetetraacetic acid (EDTA).

Serological Assays

In transplantation, serological testing has no function in detecting HCMV disease, but they are used pretransplant to establish serostatus. The serostatus of the donor and the recipient consequently indicate the post-transplant threat, considered that HCMV-negative recipients obtaining an organ from HCMV-positive donors establish much more frequent and more aggressive disease (Kotton et al, 2018). Serological diagnosis of HCMV infection can be achieved by detecting the levels of IgM and IgG antibodies. The initial antibody to appear is IgM, which may exist in the patients for a long period of time after the infection. This antibody may recur after reinfection, including of infection by different HCMV strains, exhibiting that IgM positivity is not diagnostic of a primary or recent HCMV infection. The IgG antibody is detected in the blood after 6 to 8 weeks of infection and can persist lifelong, although with variation in its levels (Chou, 1990).

Consequently, this antibody indicates the serological relationship between the donor and the recipient (D/R). If the donor's serology is inconclusive, it needs to be considered positive (Azevedo et al., 2015). If the serology is negative in the preliminary pre-transplant testing and there is a lengthy delay until the transplant, the test should be repeated,

especially if the person obtained a blood transfusion in the meantime. It is necessary to consider that the sustenance of IgG antibody does not protect a person from reactivation of a latent viral infection or from the latest infection with a various HCMV strains. Serology in immunocompromised patients can be challenging to interpret because of the patients' weakened humoral responses. Moreover, they can detect IgG derived from transfusions or from immunoglobulin therapies (Azevedo et al., 2015).

Histopathology

Histologic evaluation of tissue biopsies is useful for the diagnosis of HCMV tissue-invasive disease. Diagnosis is based on the presence of inclusion bodies, usually basophilic intranuclear inclusions, although eosinophilic cytoplasmic inclusions may likewise be seen; the diagnosis of HCMV in tissue sections can be verified with immunohistochemical staining (Kotton et al., 2013). Several experts recommend staining of biopsy samples in which inclusions bodies are not viewed as an approach to enhance the level of sensitivity (Kotton et al., 2013). Detection of HCMV inclusions in biopsy samples supports the diagnosis of tissue invasive disease.

Cellular immunity assays

Cellular assays to measure HCMV-specific immunity in immunocompromised patients are becoming a significant tool to evaluate the HCMV risk and management after SOT (Kotton et al., 2018; Ferreira et al., 2018; Meesing et al., 2019). The HCMV-nonspecific assays that measures parameters such as total lymphocyte count, CD4+ T ‑ cell matter, and nonspecific (mitogen) T ‑ cell immune responses have been correlated with the risk of HCMV disease after SOT (Meesing et al., 2019; Meesing et al., 2018). Several platforms are available to evaluate HCMV ‑ specific T ‑ cell activities such as interferon ‑ gamma release assays (IGRA) (Manuel et al., 2013; Kumar et al., 2017), enzyme linked immunosorbent area (ELISPOT) assays (Banas et al., 2018; Chanouzas et al., 2018), intracellular cytokine staining (ICS) for interferon ‑ gamma (or other cytokines) using circulation cytometry (Mihmet al., 2016; Sester et al., 2001) and major histocompatibility complex (MHC) multimer ‑ based assays that directly stain peptide ‑ specific T cells (Meesing et al., 2018). Several studies have emphasized the advantages of using cellular immunity assays in estimating HCMV risk (Manuel et al., 2013; Kumar et al., 2017; Lucia et al., 2014). Generally, the lack of satisfactory numbers of HCMV ‑ specific CD4+ and/or CD8+ T ‑ cells correlates with a higher risk of HCMV disease, therapy failure, and HCMV reactivation.

Resistance testing

Monitoring HCMV load is a valuable approach for assessing the opportunity of drug resistance. Antiviral resistance is a serious concern of HCMV disease (Lurain & Chou, 2020). Progression of resistance generally takes place after prolonged medicine exposure (>6 weeks) (Kotton et al., 2018). Patients with a constantly raised viral load, a rising viral load while on appropriate therapy, or a rebound in viral load after an initial response must be assessed for drug resistance. A rise or non-decreasing viral load during the first 2 weeks of treatment is not a dependable indication of drug resistance (Kotton et al., 2018). In the past, resistance screening was carried out by phenotypic approaches, which needed isolation of the virus in cell culture. This was a laborious procedure that took weeks to months to complete and had little effect on patient care. Currently, resistance screening is done using genotypic assays to detect certain resistance mutations by computerized sequencing techniques. The assay can identify specific mutations that cause resistance and considered as the gold standard. For instance, ganciclovir resistant HCMV is usually associated with mutation in UL97 (a protein kinase) and UL54 (a DNA polymerase) (Schott et al., 2004; Yeo et al., 2005; Noguerira et al., 2006; Liu, 2008).

Table 2. Diagnostic approaches for HCMV detection in SOT

Assays	Test Characteristics and Examples	Clinical Uses	Clinical Samples
Quantitative HCMV DNA	Detects and quantifies HCMV nucleic acid; Results reported in IU/mL	Rapid and sensitive method for diagnosis of HCMV infection; Surveillance for preemptive therapy; Monitoring of antiviral response	Whole blood and plasma
Antigenemia	Uses monoclonal antibody to detect HCMV pp65 antigen expressed in leukocytes during the early period of HCMV replication; Reported as number of pp65-positive cells per number of leukocytes counted	Sensitive diagnosis of HCMV infection; Surveillance for preemptive therapy Monitoring of antiviral response	Whole blood
Serology	Detects HCMV IgG or IgM antibodies; Various assays including complement fixation, enzyme-linked immunosorbent assay, radioimmuno-assay	Pretransplant screening of transplant candidates and potential donors for evidence of prior HCMV infection.	Whole blood, plasma, and serum
Histo-pathology	Detects HCMV antigen and cytopathic changes in tissue specimens by immunohistochemis	Gold standard for diagnosis of most end-organ HCMV diseases	Tissue biopsy

	try staining on histopathology.		
Cellular immunity assays	Measure cytomegalovirus (HCMV) cell-mediated immunity (CMI)	Assist in HCMV risk evaluation and management after SOT.	Whole blood
Resistance assay	Detects UL97 and UL54 gene mutations using automated sequencing methods directly from clinical specimens.	Management of HCMV disease related to the development of antiviral drug resistance	Whole blood and plasma

3.5. Treatment

Nowadays the therapy of symptomatic HCMV infection and disease relies on several strategies that depend on the relative immune status of the patient. Normally, intravenous treatment is recommended for patients with severe conditions or those who are undesirable with the absorption of oral agents. For SOT, first-line therapy is ganciclovir for serious illness. Based on information from documented preemptive methods, valganciclovir is currently recommended for the treatment of mild disease in paediatric SOT (Kotton et al., 2018). Other antiviral drugs, including foscarnet and cidofovir, are second-line therapies because of their hematologic toxicity and nephrotoxicity. In the possibility of marrow reductions present with ganciclovir/valganciclovir prior to transplantation, alternate agents, such as foscarnet and cidofovir can be used. HCMV Ig is not usually recommended used for the therapy of HCMV disease in paediatric SOT (Kotton et al., 2018).

Antiviral management can be empirically modified if the antiviral resistance is based on clinical suspicion, particularly in those with persistent or recurrent HCMV DNAemia with at least 2 weeks of full-dose antiviral treatment. For patients receiving ganciclovir, the options include increased ganciclovir dosages and addition or substitute to foscarnet while genetic resistance test results are pending. Results of resistance assay and certain clinical conditions such as risks for antiviral toxicity must be considered in the development of individualized antiviral therapy strategies. Maribavir, another oral antiviral agent that previously fell short in a clinical trial evaluating its usage of HCMV clearance in SOT (Winston et al., 2012) is currently under consideration for the treatment of refractory and prolonged HCMV disease in both the SOT and HSCT patients. Despite the presence of case report, Letermovir has not been studied for the treatment of HCMV disease. The rapid resistance from in vitro studies has diminished the interest for the potential of letermovir to deal with HCMV disease with high viral burdens (Chou et al., 2018). In addition, Brincidofovir has potential for the therapy of HCMV infection and disease; nevertheless, there are no recurring research intended to analyze its efficiency for HCMV in the paediatric transplant recipients.

Apart from antiviral therapy, adoptive cellular treatment with HCMV-specific cytotoxic T cells (viral-specific T-cells [VSTs] is emerging but it is mostly reported in HSCT. HCMV VSTs are predominantly originated from either the donor or a third party, although production from naïve T cells from umbilical cable contributors has been performed. Treatment by HCMV VSTs on refractory and prolong HCMV disease in HSCT recipients has been successful (Peggs et. al. 2011). The progress of GVHD because of mismatch third-party human leukocyte antigen (HLA) which may reduce the efficiency of the treatment has not been reported (Neuenhahn et al., 2017). Although the use of HCMV VSTs in SOT is uncommon, administration in adult lung and kidney transplant described in case records has been effective (Brestrich et al., 2009; Macesic et al., 2015). More studies are required to evaluate the unanticipated adverse effects of HCMV VSTs therapy.

3.6. Prevention

The thought of HCMV infection and disease are frequently concerning about donor-derived acquisition or reactivation of latent infection in transplant recipients or oncology patients. However, immunocompromised children who are HCMV negative are also at risk of getting primary HCMV from community exposures. Primary HCMV infection in immunocompromised children may have greater risk of developing symptomatic disease and may be related to graft rejection in transplant recipients (Ramanan & Razonable, 2013). Education of patients and households concerning to HCMV exposure and prevention methods for children who are HCMV negative is an essential part of regular peritransplant counselling to prevent HCMV infections and more secure living post-transplantation. Immuno-compromised transplant recipients, oncology patients, and other children should receive HCMV-negative or leuko-reduced blood products to prevent transmission of HCMV, especially in patients who are previously HCMV negative patients.

4. CONCLUSION

HCMV is an important pathogen in immunocompromised individuals, particularly those undergoing SOT or HSCT. Many improvements have been made in diagnostic approaches, treatment of HCMV disease, and techniques for screening and prevention towards the patients who are susceptible to primary HCMV acquisition while immunosuppressed. New antiviral drugs for prophylaxis and treatment, immunomodulatory strategies, and advancement of vaccines will eventually be needed to curb the burden of HCMV infection and disease in immunocompromised especially in paediatric populations. Expansion of research efforts remains a significant need to discover new strategies on HCMV management especially in transplant setting.

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