

Pathogenicity and Diagnostic Methods of Human Cytomegalovirus

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ABSTRACT

HUMAN CYTOMEGALOVIRUS (HCMV) IS A BETA-HERPESVIRUS CAPABLE OF ESTABLISHING LIFELONG INFECTION IN HUMANS. It is primarily transmitted through bodily fluids like blood, urine, and saliva. In healthy individuals, primary HCMV infections are often asymptomatic but can lead to persistent or latent infections. *However,* immunocompromised individuals such as organ transplant recipients and those with acquired immunodeficiency syndrome, are at risk of severe and potentially fatal illnesses resulting from HCMV pathogenicity. Consequently, it is crucial to gain a comprehensive understanding of HCMV's pathogenicity and diagnostic methods, particularly for detecting the presence of HCMV in individuals with compromised immune systems. This review aims to address these aspects and provide insights to assist relevant authorities in designing effective interventions and managing HCMV-related illnesses.

Keywords: diagnostic methods, human cytomegalovirus, infectious diseases, pathogenicity

INTRODUCTION

Cytomegalovirus (CMV) infection is a highly prevalent contagious disease caused by a type of herpes virus. It can infect a significant portion of the global population, ranging from 45% to 100%, depending on socioeconomic factors (Cannon et al., 2010). CMV belongs to the *Herpesviridae* family and possesses a double-stranded DNA genome. The human cytomegalovirus (HCMV) specifically targets humans among the different species of CMV. HCMV is classified as a beta-herpesvirus that establishes lifelong infections in humans. Several common risk factors associated with HCMV infection include being female, older age, low socioeconomic status, and weakened immunity (Stockdale et al., 2018; Fowler et al., 2022). HCMV is primarily transmitted through bodily fluids such as blood, urine, and saliva, with salivary and sexual contact being the main routes (Jackson & Sparer, 2018; Sezgin et al., 2019).

In healthy individuals, primary HCMV infections typically do not exhibit symptoms, but they can result in persistent or latent infections. However, the virus can have significant pathogenicity in individuals with compromised immune systems, leading to severe and occasionally fatal illnesses. Interestingly, intrauterine HCMV infection can have detrimental effects on foetal health, resulting in bone abnormalities, hearing loss, hepatosplenomegaly, microcephaly, and varying degrees of mental retardation (Swanson & Schleiss, 2013). HCMV also poses serious risks to organ transplant recipients and individuals with acquired immunodeficiency syndrome (AIDS), whether it originates from primary infection or reactivation of a latent infection. Complications such as HCMV retinitis, pneumonia, and gastrointestinal disorders including gastroenteritis, hepatitis, and colitis are commonly observed in organ or bone marrow transplant recipients and immunocompromised individuals, particularly those with AIDS (Bhat et al., 2015; Ong et al., 2022). More frighteningly, a recent meta-analysis has revealed an association between HCMV infection and the development of gastrointestinal cancer in humans (Lv et al., 2020). Currently, there are limited antiviral drugs available for treatment and preventing HCMV infections.

Considering the severe diseases caused by HCMV in humans, it is essential to comprehend its pathogenicity and diagnostic methods. Comprehending the pathogenic nature of HCMV holds the utmost importance in the development of therapies and the prevention of infections. Nonetheless, familiarity with various diagnostic approaches of HCMV could pave the way for swift and accurate diagnosis, enabling effective treatment and enhanced patient cases. This review provides a comprehensive discussion of these aspects, intending to assist relevant authorities in managing public health associated with HCMV appropriately.

PATHOGENICITY OF HUMAN CYTOMEGALOVIRUS

The ability of different microbes to induce disease is referred to as pathogenicity or virulence. This encompasses their capacity to replicate within the host's tissues, infiltrate and enter the host, interact with or evade the host's immune defences, cause disease, and transmit between hosts. Due to the complex nature of its infection pattern, HCMV cannot be simply classified as virulent, attenuated, or avirulent. Primary HCMV infections generally do not produce noticeable symptoms, but they can progress to persistent or latent infections. The pathogenicity of the virus becomes evident primarily in individuals with compromised immune systems (Ong et al., 2022). Hence, HCMV is considered an opportunistic pathogen that can evade the host's immune response and reactivate to cause disease. Its pathogenicity depends on multiple factors, including viral load and the immunological condition of the host (Griffiths & Reeves, 2021). Furthermore, in addition to its ability to infect various cell types, HCMV's capacity to persist within the host even in the presence of a fully functioning immune system contributes to its widespread prevalence.

The determination of nucleotide sequences in HCMV strain AD169 has provided researchers with valuable insights into the biological significance of various viral genes (Schleiss, 2011; Wilkinson et al., 2015). A comprehensive review of the HCMV's genome suggests that many of the viral genes play an essential role in the pathogenesis of the virus (Schleiss, 2011). Interestingly, numerous studies indicate that the immune response is crucial in controlling HCMV infection, with key immune cells such as natural killer cells and T cells, along with important cytokines like tumour necrosis factor, interleukins, and interferons, have been shown to have critical functions in this process (Schleiss, 2011; Rosa & Diamond, 2012). However, the mechanisms of how these immune cells and cytokines control HCMV infection remain unclear.

A fundamental characteristic of HCMV is its ability to establish a lifelong latent infection in the host, which can lead to severe and even fatal diseases upon reactivation. Given the significant impact of the virus on cell-mediated immunity, it is reasonable to assume that HCMV could contribute to overall mortality, especially in individuals with compromised immune systems. Griffith et al. (2015) hypothesized that the mechanisms driving latency are activated after viral entry rather than being a result of using alternate receptors. An important step in establishing latency is the regulation of viral lytic gene expression through the silencing of the viral main immediate early promoter (Collins-McMillen et al., 2018). The absence of lytic gene expression during latent carriage of the virus represents one of the most notable distinctions between viral latency and reactivation. In healthy individuals who carry the virus, reactivation from latency can occur frequently but is often constrained by a robust immune response. However, in immunocompromised individuals, reactivation from latency serves as a well-known source of morbidity and mortality.

Regarding the latent reservoirs of HCMV, peripheral blood monocytes have emerged as a crucial cell population for *in vivo* HCMV latent infection (Elder et al., 2019). A comprehensive analysis of the peripheral blood compartment has revealed the significant role of monocytes in carrying HCMV within the host. Moreover, HCMV latency sites have been identified in hematopoietic stem cells within the bone marrow, specifically in undifferentiated myeloid cells rather than cells of the lymphoid lineage (Wills et al., 2015; Elder et al., 2019). While the myeloid lineage represents a confirmed site of true latency for HCMV, there may still be other undiscovered locations of *in vivo* latency. Therefore, utilizing a sensitive and specific diagnostic method for HCMV detection is undoubtedly crucial.

DIAGNOSTIC METHODS FOR HUMAN CYTOMEGALOVIRUS

While severe diseases caused by HCMV are rare in individuals with a healthy immune system, immunocompromised individuals, such as organ transplant recipients, AIDS patients, and premature newborns, are at greater risk and may experience a wide range of complications due to HCMV. Therefore, accurate diagnostic tests are essential for effectively managing HCMV infection and associated illnesses in humans. Various methods are available to detect the presence of HCMV, including serology, antigenemia, immunohistochemistry, cell culture, hybrid capture assay, polymerase chain reaction, and nucleic acid sequence-based amplification. Table 1 provides a comprehensive overview of the advantages and disadvantages of these diagnostic methods for HCMV detection.

Table 1 Advantages and disadvantages of diagnostic methods for HCMV detection

Methods	Advantages	Disadvantages
Serology	<ul style="list-style-type: none"> Determine current infection and past exposure by detecting antibodies. High-throughput capacity and suitable for large-scale screening. 	<ul style="list-style-type: none"> Not able to distinguish between active and latent infection. False negatives can occur, particularly in immunocompromised individuals with impaired antibody response.
Antigenemia	<ul style="list-style-type: none"> Identification of active infection by targeting viral antigens. Provides quantitative information on viral load and is therefore useful in monitoring response to antiviral therapy. 	<ul style="list-style-type: none"> Requires trained personnel and specialized laboratory equipment. Longer turnaround time thus not suitable for rapid diagnostic.

Cont.

Methods	Advantages	Disadvantages
Immunohistochemistry	<ul style="list-style-type: none"> Useful to identify HCMV in biopsy specimens. Essential in diagnosing HCMV-related diseases, such as organ transplant-related complications. 	<ul style="list-style-type: none"> Requires skilled personnel for collecting biopsy specimens, sample processing, and data interpretation. Limited sensitivity compared to the molecular method and longer turnaround time.
Cell culture	<ul style="list-style-type: none"> Able to provide essential information on viral tropism and drug susceptibility. Allows viral isolation and propagation for further characterization of the virus. 	<ul style="list-style-type: none"> Requires specialized laboratory facilities and expertise. Time-consuming as the results take days to weeks to obtain.
Hybrid capture assay	<ul style="list-style-type: none"> Allows the detection of viral DNA and RNA simultaneously, thus quantitative information can be obtained. Capable of screening large numbers of samples. 	<ul style="list-style-type: none"> Higher cost compared to some other methods. Requires specialized equipment and reagents for detection.
Polymerase chain reaction	<ul style="list-style-type: none"> Highly specific and sensitive method for the detection of HCMV. Fast turnaround time with results available within hours. 	<ul style="list-style-type: none"> Requires trained personnel and thermal cycler for detection. Relatively higher cost compared to some other methods, especially the detection using a real-time PCR approach.
Nucleic acid sequence-based amplification	<ul style="list-style-type: none"> Highly sensitive for detecting HCMV RNA. Quantitative measurement of viral load can be obtained, thus useful for monitoring response to antiviral therapy. 	<ul style="list-style-type: none"> Limited availability in some settings, especially in rural areas. Relatively higher cost and requires specialized equipment.

Serology

Serological tests are the most commonly used method to determine a patient's previous HCMV infection by assessing the presence or absence of HCMV IgG and IgM antibodies. Various assays, including enzyme-linked immunosorbent assay, anticomplement immunofluorescence, radioimmunoassay, indirect hemagglutination, and complement fixation, have been developed and evaluated for HCMV antibody detection (Razonable et al., 2020). A recent systematic literature review has shown that many countries worldwide utilize this method for the detection of HCMV (Fowler et al., 2022). However, it is important to note that IgM assays lack specificity as the presence of HCMV IgM alone cannot solely diagnose primary infection (Ross et al., 2011). IgM antibodies can persist in the body for months following primary infection and can also be present during secondary or reactivated HCMV infection, which can lead to potential false-positive results. To overcome this limitation, IgG avidity assays are commonly

employed alongside IgM detection to distinguish between primary HCMV infection and reinfection. While serology offers a swift method for diagnosing HCMV infection, it is essential to interpret the results accurately due to the elevated occurrence of false positive IgM antibody tests for HCMV (Iijima, 2022). This is particularly notable in patients with concurrent autoantibodies associated with lupus (Cannavan et al., 1998). Hence, it is advisable to complement serological tests with alternative diagnostic methods to ensure precise results.

Antigenemia

The antigenemia test is a direct approach used to detect active HCMV infection in blood samples and has been widely employed for quantifying HCMV virus levels. The test relies on immunocytochemistry to identify HCMV immediate early antigens in blood leukocytes. It utilizes monoclonal antibodies to identify the viral pp65 antigen, which is a structural late protein expressed in blood leukocytes during the early phase of HCMV replication (Ross et al., 2011; Carstensen et al., 2019). The level of HCMV antigenemia is directly linked to the clinical severity of the disease but is inversely related to the host's immune competence. This test offers both qualitative and quantitative results but can only detect the virus present in leukocytes. It is valuable for early and prompt diagnosis of life-threatening HCMV infection in immunocompromised patients and for studying the immunopathophysiology of HCMV disease (Carstensen et al., 2019). However, this method has certain limitations. It is labour-intensive and has a low throughput. Accurate test performance and precise result interpretation require highly skilled personnel. Additionally, samples must be processed immediately within 6 hours, as any delay significantly reduces the sensitivity of the test (Schafer et al., 1997).

Immunohistochemistry

Immunohistochemistry (IHC) is a method that utilizes antigen-specific antibodies to identify specific antigens in tissue or bodily fluid samples. By conducting IHC studies on the detection of HCMV intranuclear inclusions, HCMV infection or reactivation can be diagnosed (Touma et al., 2021; Goyal et al., 2022). This technique involves the use of portions of biopsy tissue samples or centrifuged cells to create a slide. To detect the presence of HCMV, monoclonal antibodies against HCMV antigens are applied to the slide and visually observed using fluorescently tagged antibodies or enzyme-labelled secondary antibodies that produce colour changes. The stained slides are then analyzed using fluorescence or light microscopy. However, it is a time-consuming process that requires trained personnel to collect biopsy specimens, process the specimens, and accurately examine the slides. The application of IHC in detection could elevate the likelihood of post-biopsy complications, especially in immunocompromised individuals (Cleveland et al., 2019). As a result, a thorough assessment by considering the patient's clinical manifestations is warranted to determine the appropriateness of employing this method for HCMV detection.

Cell Culture

Conventional cell culture is considered the traditional method for HCMV detection, involving the application of samples onto MRC-5 human fibroblast cells (Hematian et al., 2016). In this technique, the culture is incubated and observed for 2 to 21 days to monitor the cytopathic effect. The cytopathic effect of HCMV is characterized by the presence of foci of flat and enlarged cells, which correlates with the virus titer (Ross et al., 2011). The shell vial assay is an improved version of the conventional viral culture method, offering rapid *in vitro* HCMV detection (Storch, 2000). This approach utilizes MRC-5 human fibroblast cell cultures inoculated on coverslips within a shell vial culture tube. The tube is centrifuged and incubated for a specific duration. By centrifuging the specimen onto the cell monolayer, the adsorption of the virus is enhanced, thus increasing its infectivity to susceptible cells. The low-speed centrifugation mechanically stresses the cell surface, facilitating virus entry and reducing the overall infection time. After incubation, viral antigens can be detected using monoclonal antibodies targeted at the HCMV immediate-early viral antigen through indirect immunofluorescence. This assay has gained prominence in clinical virology due to its sensitivity and allows viral isolation and propagation for further characterization of the virus (Dolskiy et al., 2020; Schwartz et al., 2023).

Hybrid Capture Assay

The hybrid capture assay (HCA) is a quantitative DNA hybridization test used to detect HCMV DNA in leukocyte blood samples. This assay employs RNA probes to identify and quantitatively measure the HCMV DNA. HCA enables early diagnosis and monitoring of antiviral therapy in AIDS patients and solid organ transplant recipients (Abu-Nader & Patel, 2000). Although HCA allows the simultaneous detection of viral DNA and RNA for quantitative purposes, it is less commonly used for HCMV detection due to its higher cost compared to other methods. Additionally, specialized equipment and reagents are required for the detection. Nevertheless, a previous study empirically demonstrated that while HCA is more sensitive than the blood culture assay in HCMV detection, it is considerably less sensitive than molecular approaches such as the polymerase chain reaction (Hebart et al., 1998). Hence, the outcomes derived from HCA necessitate additional validation through more reliable methods.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a widely used and rapid technique for detecting HCMV infection through nucleic acid amplification. DNA samples from various sources such as whole blood, tissue, saliva, and urine can be extracted for analysis (Ross et al., 2011; Razonable et al., 2020). PCR allows both qualitative and quantitative measurements, estimating the quantity of HCMV DNA present in the samples. In HCMV DNA detection, PCR commonly targets conserved regions of major immediate-early and late antigen

genes, although other genes are also utilized (Haynes et al., 2013). Besides standard PCR, real-time PCR also can be employed, which provides a precise means of quantifying HCMV DNA. Many studies have reported the use of real-time PCR in HCMV detection and quantification in various sample sources with promising results (Gault et al., 2001; Ligozzi et al., 2016; Fernandes et al., 2021), and it is a more sensitive and reliable method compared to enzyme-linked immunosorbent assay (Enan et al., 2011; Ali et al., 2019). Due to the availability of the different fluorescent probes, several multiplex real-time PCR systems have been developed to detect the variant of the target gene in HCMV DNA and simultaneously detect the presence of other viruses in transplant patients with high accuracy (Zawilinska et al., 2016; Hwang et al., 2018). Currently, PCR stands as the most favoured approach for detecting HCMV due to its high sensitivity and accuracy. Additionally, its widespread availability across laboratories enables prompt diagnosis, facilitating effective patient treatment.

Nucleic Acid Sequence-Based Amplification

The nucleic acid sequence-based amplification (NASBA) assay is a highly sensitive transcription-based amplification method that operates under isothermal conditions. It allows specific amplification of unspliced viral mRNAs, such as immediate early mRNA and late pp67 mRNA expression, even in the presence of a DNA background. This technique has been successfully applied for the qualitative detection of HCMV, enabling improved differentiation between active and latent infections. It has also been utilized to detect congenital HCMV infection in amniotic fluid with high sensitivity and specificity (Revello et al., 2003). Furthermore, a previous study has reported that NASBA is preferred over the antigenemia assay for monitoring HCMV infection during antiviral therapy due to its sensitivity in HCMV detection (Blok et al., 2000). The NASBA procedure follows standardized protocols and can be completed within a day. However, it is important to note that mRNA extraction in the NASBA process can be labour-intensive. Additionally, the availability of NASBA for HCMV detection is limited as it requires specialized instruments, and the associated cost is relatively high.

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, HCMV infection can lead to severe and fatal illnesses in transplant recipients and immunocompromised individuals. Additionally, identifying essential genes involved in the pathogenesis of HCMV could serve as potential targets for vaccine development. At present, multiple vaccines are undergoing preclinical, early clinical, and advanced clinical development. However, Phase III data is not yet accessible (Plotkin et al., 2020). Also, a majority of these vaccines are aimed at the glycoprotein B gene of HCMV. Therefore, maintaining continuous genetic surveillance of this gene is recommended to ensure the future efficacy of the vaccines. Subsequent research could also delve into the possibility of utilizing genetic engineering tools like zinc finger nucleases and clustered regularly interspaced short palindromic repeats (CRISPR)-

CRISPR-associated protein 9 system for HCMV treatments, given the absence of existing clinical data in this area. Several approaches are available for HCMV detection, each with its distinct advantages and disadvantages. It is the responsibility of the relevant authorities to carefully select the most suitable methods for detecting the presence of HCMV in individuals with compromised immune systems to ensure effective treatment.

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