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CYTOMEGALOVIRUS

Cytomegalovirus (CMV) is a lytic, double stranded DNA virus belonging to the Betaherpesvirinae subgroup of the Herpesviridae family. It is widespread and thought to infect approximately 40% of adults worldwide. In common with other Herpesviridae viruses, CMV persists for life in a latent state following primary infection. However, it is possible for latent CMV to become reactivated, allowing shedding of infectious virus in bodily secretions such as urine and saliva. The presence of CMV in bodily secretions allows it to spread from person to person through close physical contact. It can additionally be transmitted through sexual activity and organ transplants, as well as through blood transfusions, although this is rare. CMV can also be spread from mother to baby through placental infection, maternal genital secretions during delivery and via breast milk.

Symptoms

In immunocompetent young children and adults, both primary and recurrent CMV infections are typically asymptomatic. In individuals who do develop symptoms, they tend to resemble those of mild flu and normally last for no longer than two weeks. In contrast, CMV infection can be problematic in immunocompromised patients and unborn or prematurely born babies whose immune system is not yet fully developed (congenital CMV). In extreme cases, CMV infection of the unborn baby can result in a stillbirth. In babies born with congenital CMV, symptoms can include jaundice, pneumonia, enlarged liver and spleen, low birth weight and seizures. They may also develop long-term problems including hearing loss, visual impairment, learning difficulties and epilepsy.

In immunocompromised individuals, symptoms are widespread and can vary across patient groups. Disease manifestations that may be evident include fever, pneumonia, hepatitis, gastrointestinal problems, retinitis and neurological symptoms. Bone marrow transplant patients with CMV often develop pneumonitis, whilst hepatitis is commonly observed in those with liver transplants. In contrast, retinitis is a frequent manifestation in CMV infected individuals with AIDS, although this has become less prevalent since the introduction of highly active antiretroviral therapy (HAART).

Diagnostic tests and their interpretation

Serological testing

A variety of tests can be used in the diagnosis of CMV. These typically involve either detecting an immune response to the virus (serological tests), or the detection of the virus itself (PCR). Serological testing can allow identification of either past or recent CMV infection. A number of tests are available for detecting both CMV IgG and CMV IgM antibodies. The CMV IgG antibody is produced in the early stages of primary infection and persists for life. It is therefore a useful test for determining an individual's CMV immune status, for example in organ donors and patients with immunodeficiency. Additionally, rising IgG antibody levels can indicate recurrent infection. CMV IgM antibodies are also produced in the early stages of primary infection. However, in contrast to the IgG antibody, it is only detectable in the first 3 – 4 months of infection and is not necessarily present in cases of viral reactivation. Detection of IgM antibody is therefore useful in cases of recent symptomatic primary infection.

CMV DNA detection

The use of molecular amplification processes provides a rapid and sensitive method for detecting CMV DNA. A number of commercially produced kits exist which can be performed on a variety of real-time PCR platforms. In-house assays have also been developed, which are largely based on real-time PCR. Micropathology Ltd performs both qualitative and quantitative CMV DNA detection using an in-house probe-based molecular amplification assay. This assay is included in external quality assurance schemes.

UKAS accredited sample types for CMV in the Screen 2 assay include: CSF, plasma, serum, EDTA whole blood, and urine for quantitative detection, and amniotic fluid, bronchoalveolar lavage samples (BAL) and saliva samples for qualitative detection only. If samples are not homogenous, for example cell clumps in whole blood, stochastic differences in the input material during DNA extraction might lead to differences in quantitative results, especially in low viral loads. EBV has been successful detected in other sample types, such as biopsies, swabs, and tissues, however, validation of these sample types has not been performed. However, processing of non-UKAS-accredited sample types can still be performed, but will contain the caveat: "Assay for this organism is not UKAS accredited for this sample type."

CMV drug resistance testing

Ganciclovir (GCV) is the most widely used antiviral therapy for CMV and has proved to be effective in reducing the CMV viral load in organ transplant recipients

and patients with AIDS. As a result, this has led to the development of GCV-resistant CMV strains. Factors increasing the risk of a patient developing GCV resistant strains include donor-positive, recipient-negative organ transplant recipients, prolonged antiviral therapy (over 3 months), increased immunosuppression, severe CMV, high viral loads and lung transplantation. The majority (90%) of GCV resistant CMV strains possess *UL97* gene mutations. Less commonly, strains may possess mutations in the *UL54* gene, and in these circumstances the virus may also be cross-resistant to alternative therapies such as cidofovir and in a minority of cases to foscarnet. In 2023 maribavir was approved for anti-CMV viral therapy and resistance mutations in the CMV *UL97* gene were soon reported.

The sequencing of genes allows resistant mutations to be identified and is therefore useful for determining the appropriate treatment. This can include increasing the dose of GCV or switching to an alternative antiviral such as foscarnet.

NICE have recently approved the use of letermovir in certain cases for the treatment of CMV in transplant patients with known resistant virus, on compassionate grounds. The drug targets the CMV tripartite Terminase complex (consisting of proteins from UL51, UL56 and UL89).

Currently, UL56 mutations at amino acid position C325 have been shown to confer significant levels of resistance against letermovir, both *in vitro* and *in vivo*. UL89 is not currently considered to have clinically important resistance mutations, but in vitro studies have shown that mutations do occur against letermovir selective pressure and may become clinically significant over time. UL51, the remaining component of the tripartite terminase complex has only been shown to develop letermovir associated mutations *in-vitro*.

At Micropathology Ltd we use next-generation sequencing (NGS) for screening for drug resistance mutations the CMV genome. This provides improved detection of mutations when in low abundance, down to 5%, in a heterogeneous (mixed) population of viruses compared with the previously used Sanger sequencing methods where we were typically able to detect mutations at a frequency greater than 10-20%. In addition, the enhanced capability of NGS enables us to include in our reports the sub-population percentage of drug resistance mutations. We perform sequencing of CMV ganciclovir resistance testing for *UL97* and *UL54* encoded resistance mutations, as well as cidofovir/foscarnet *UL54* mutations and *UL56/UL89* testing for letermovir drug resistance mutations. Testing for mutations in *UL97* associated with maribavir resistance is currently offered on a research-only basis.