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Cytomegalovirus Infection in Liver Transplantation

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1. Introduction

With the global evolution of organ transplantation in humans a new class of patients with special problems related to opportunistic infections after transplantation has appeared [1, 2]. Some of these challenges infections with members of the herpesvirus family. Among these viruses, human cytomegalovirus (HCMV) often affects immunocompromised patients, HCMV can be reactivated by immunosuppression and cause significant morbidity and mortality [3,4]. In the postoperative period, HCMV infection can result in serious complications in patients who received grafts by modulating the immune response [5]. However in immunocompetent individuals cytomegalovirus infection can be asymptomatic or cause symptoms similar to infectious mononucleosis syndrome, such as lymphadenopathy, fever, rash, malaise, arthralgia, hepatomegaly and splenomegaly [6].

This chapter presents the main clinical and epidemiological aspects related to cytomegalovirus infection and the importance of detection in liver transplant recipients.

2. Cytomegalovirus history

The discovery of HCMV began in 1881 when the histological effects of infection were observed in the kidney of a newborn child. In 1904, Ribbert identified the causative agent of "cytomegalic inclusion disease", whose name derives from the characteristic cytopathic effect, represented

by increases in cell volume and intracellular cytoplasmic inclusions in infected tissues [7]. In 1881 and 1921 similar cell characteristics were reported by Goodpasture and Talbot in a fatal case associated with this virus involving lung, liver and kidney from a newborn child [8].

The first experimental evidence of the likely etiologic agent of "cytomegalic inclusion disease" was proposed by Cole and Kuttner in 1926, when they demonstrated the transmission of the disease in guinea pigs and suggested that this agent possessed characteristics of viral infection and was species-specific. Wolbach and Farber (1932) demonstrated the first evidence that the salivary gland virus was commonly involved and showed typical cytomegalic cells were found in 12% of children. In 1954, using the salivary infection mouse model, Smith isolated the virus in tissue culture [9].

In 1970 study groups were organized to evaluate the impact of infection in immunocompromised people and through this to propose infection control strategies. In the 1980s the control measures of CMV began with antiviral agents and immunological interventions [10].

3. Structure features and replication engine

CMV has an ultrastructure similar to other herpesviruses with four structural elements: an electron-dense core, an icosahedral symmetry capsid, a tegument occupying space between the capsid and an envelope steeped in glycoproteins and membrane proteins [11].

CMV carries a double-stranded DNA containing approximately 240 kb linear bases [12] encoding 33 structural proteins and an indefinite number of non-structural proteins, some of which are antigenic. The genome can be divided into two segments, designated as long component (L) and short (S) defined by repetitive sequence terminals (RT). The CMV has a complex genome due to the acquisitions of host genes and the duplication of viral genes [13]. It is a very thermolabile virus and its average life at 37 ° C is only 45 minutes, totally inactivated at 56 ° C for 30 minutes [14].

During natural infection, viral replication can occur in epithelial, endothelial and muscle mesenchymal cells, hepatocytes, granulocytes and macrophages [15, 16]. In vivo studies with cells from immunocompetent and immunosuppressed patients show that CMV can commonly be isolated from polymorphonuclear leukocytes [17], which may represent an important replication site [14,16]. Variants of CMV are found in mice, monkeys and guinea pigs, but these strains are species-specific and do not infect humans [14].

The CMV replication mechanism occurs in three distinct stages, similar to other herpesviruses. The early phase occurs when the virus adheres to the host cell membrane (with the envelope loss and penetration into the cell), in the intermediate phase the gene expression and genome replication occur, and in the late phase, there is the assembly and release of new viral particles [16-18].

The "early" phase begins when surface proteins of the virion adhere to specific protein receptors on the cell surface through non-covalent bonds. The viral particles penetrate by

endocytosis, entering pinocytic vesicles in which the envelope loss process is started, favored by a low pH. The rupture of the vesicles or fusion of the virus with the outer layer of the vesicle membrane deposits the core of the virus in the cell cytoplasm [13].

The intermediate phase lasts 24 hours, characterized by transcription and replication of viral DNA. The first step of viral gene expression is the synthesis of mRNA via host RNA polymerase inside the core. The mRNA is translated by the host ribosomes into early and late viral proteins. The early proteins are enzymes required for viral genome replication [19]. The late proteins include a polymerase replicating the viral genome [13].

In the late phase, viral particles newly formed are grouped together within the capsid and begin the process of budding, during which the nucleocapsid adheres to specific sites of the membrane and interacts with the protein matrix. At this point, a process of evagination occurs and an enveloped particle flows from the membrane surface [13].

4. Transmission and epidemiology

Infection is defined as seroconversion (an increase of 4 times or more in HCMV antibody titer in seronegative or seropositive patients), virus circulation in any body fluid such as urine, nasopharyngeal secretions or blood [7]. CMV infects only the human population and its transmission occurs both horizontally and vertically and may include oropharyngeal secretions, vaginal tears, seminal fluid, breast milk, urine, feces and blood [19]. In adulthood the CMV transmission may occur through heterosexual and homosexual contact, through blood and blood products and through organ transplantation, the latter being an important route of transmission [12,19, 20].

About 80% of the population between late childhood and early adolescence is already infected with CMV [21, 22] and can harbor the virus in various body sites, especially in the salivary glands and different types of leukocytes. With age the increased prevalence of antibodies is common. This may not depend on the geographical area, but the socio-economic status may be important [10, 23- 28]. The seroprevalence of CMV in populations at high socioeconomic level varies from 40% to 60%, increases after infection of early childhood and approximately 10% to 20% of children have their first infection episode before puberty [10]. In lower socioeconomic populations the seroprevalence level is higher, ranging from 80% to 100%. In Brazil, seroprevalence of cytomegalovirus averages 90% in adult populations [28].

5. Clinical manifestations

The clinical course of CMV in immunocompetent individuals may be asymptomatic or may resemble "Mononucleosis Syndrome" presented by persistent fever, myalgia, pharyngitis, lymphadenopathy, sweating and hepatosplenomegaly [10,28-34].

After primary infection, CMV persists in host tissues and may be reactivated to cause disease – usually in children with congenital infection, organ transplant recipients, cancer patients undergoing chemotherapy and patients with HIV disease [7,10,29,35].

Among the complications caused by CMV in transplanted patients increased long-term mortality and worsening graft survival are common [35-37]. Clinical disease caused by CMV is expressed by fever, malaise, myalgia, leukopenia (WBC less than $4.0000/\text{mm}^3$), increased transaminases (hepatitis), pulmonary (pneumonitis) and/or gastrointestinal (colitis, gastritis, esophagitis) and fever being the most common manifestation, which can also occur with neurological symptomatology compatible with encephalitis but these are rarer [29,38,39].

Clinical disease may reflect

1. Primary infection, when it occurs in patients previously seronegative;
2. Secondary infection occurs when the reactivation of latent infection or superinfection;
3. Tertiary infection by reinfection by other strains of the virus [7].

The source of infection for both primary infection and superinfection is may include graft and blood transfusions. Immunosuppression may cause reactivation of CMV [40]. About two thirds of patients with primary infection are symptomatic, less than 20% in viral reactivation have symptoms and about 40% of reinfected individuals have symptoms attributable to CMV [10]. When primary infection occurs after transplantation the clinical impact is significant [41]. This is most common following allocation of grafts from donors with positive serology to seronegative recipients [42]. Immunosuppressive drugs such as azathioprine and cyclosporine have been implicated in the facilitation of CMV disease [7].

Diagnostic criteria include clinical signs known to be caused by this virus [43,44]. In liver transplant patients with active CMV infection, about 80% will develop clinical manifestations of the disease and the rate may be higher when the recipient is seronegative and a donor is seropositive [44]. CMV infection is an independent risk factor for the development with opportunistic infections, as well as graft rejection [7]. The evidence of viral replication and clinical symptoms in transplant occurs mainly during the 1st to 4th month post-transplant.

The most common clinical manifestations are interstitial pneumonia, esophagitis, gastritis, colitis, retinitis, fever and delayed engraftment in bone marrow transplants [29,45]. During liver transplantation, primary infection tends to be more important as the CMV viremia may be limited to when virus replication is detected in peripheral blood or significant increase of specific antibodies without symptoms or viral syndrome presenting fever equal or greater than 38°C , malaise, leukopenia, atypical lymphocytosis equal or less than 3-5% and thrombocytopenia [14, 37, 42, 45,46].

Antiviral treatment controls the acute manifestation of the disease in most cases, but may not eradicate the CMV with recurrence reported in 26-31% of solid organ transplant recipients.. [14, 36].

6. Diagnosis

The diagnosis of CMV infection can be done by serology, polymerase chain reaction (PCR), culture and viral antigenemia research. Early diagnosis is important as early treatment of asymptomatic active infection reduces morbidity [24, 31, 46, 47]. The first method of diagnosis used to identify the CMV was exfoliative cytology. This technique revealed the presence of large cells which had inclusions within the core, identified as cytomegalic inclusion. Later methods are more sensitive and specific. These are grouped into seven categories: cytological, histological, virus isolation, serological identification,, **Immunofluorescence, detection of viral antigens** and molecular methods [49, 48]...

Cytopathological techniques: These methods can be performed on tissue and secretions aspirated material [7,8], but have low sensitivity so they currently have little use in clinical practice.

Histological Techniques: A method of detecting inclusions by visualization of typical tissue. The finding of cells with typical inclusions allows often to correlate CMV lesion or dysfunction of the organ studied. Although this method has low sensitivity, it reveals invasive tissue disease [7]. The advantages are low cost, simplicity of use and availability of equipment.

Viral Isolation: CMV can be isolated from various biological materials, such as biological fluids (urine, saliva, blood, cervical secretions, breast milk, tears, semen, feces and washed aspirates organs) and tissue obtained from biopsies or autopsies [49, 50].

Serological methods: The modern serological methods detect the presence of IgM and IgG [51] usually by ELISA. This technique does not detect the virus in early stages of infection, as antibodies are produced by the host only after this phase.

Immunofluorescence (IF): A method that allows an early detection of the virus is immunofluorescence usually using commercially available antibodies.

Detection of Viral Antigens (antigenemia): Antigenemia offers high sensitivity and specificity. It is fast, direct and sensitive, and is considered a quantitative technique for viral load [54, 55]. CMV antigenemia is one of the earlier tests with positive results [17, 25, 51- 58] and becomes positive on average 9-18 days before establishment of the disease. It has been widely used for the early detection of active infection in organ transplant recipients [17, 24, 25, 36, 56].

The additional advantage of this method is that results can be expressed quantitatively, allowing observation of the clinical response to treatment [17, 59]. The disadvantage of this technique is the speed needed to process the collected material without loss of sensitivity - 6 to 8 hours [17]. In patients with neutropenia, this test cannot be performed due to low granulocyte count. On this situation, molecular assays are used.

Molecular methods

Polymerase chain reaction-PCR: The qualitative PCR is often the first test to detect asymptomatic subclinical infection, but specific predictive value is low for the diagnosis of HCMV

disease. It is not suitable for routine monitoring of patients on treatment [57]...why not???. It is a quick (4-6 hours), specific and extremely sensitive test but false positives may result from contamination during the test run. False negative results can also occur due to presence of inhibitors in the sample [55,60]. The sample type and method of extracting DNA from these samples must be carefully chosen to avoid this [31,61].

Nested PCR (Polymerase Chain Double): Nested PCR (N-PCR) has been used to increase the sensitivity and specificity of simple PCR. Here the product of the first PCR, amplified with a primer pair, is subjected to a new amplification reaction using another pair of primers internal to the first, the product being then detected by agarose gel electrophoresis [62]. Nested PCR technique to diagnose CMV infection produces results consistent with classical culture, reaching 100% specificity and 93% sensitivity in a shorter time frame [63- 66].

Real-time PCR: Real Time PCR amplification (RT PCR) presents high sensitivity and precision. It has been used for the detection and monitoring of viral load. Its sensitivity and specificity are directly related to the choice of "primers" and probes, and the accuracy is determined by the threshold cycle, which is calculated during the exponential phase of the reaction. Formation of a fluorescently labeled product is monitored at each amplification cycle in a single instrument generating quantitative results. [58].

7. Treatment

Ganciclovir has been the "gold standard" for treatment of CMV disease although resistance to this drug has been reported and should be considered in unresponsive patients. Some studies have focused on genotyping of CMV that could indicate samples that were resistant to conventional treatment. Inadequate dosing may reduce clinical efficacy and promote resistance (44)

Antiviral administration is generally initiated in the immediate or early post-transplant period, and continues for 3 to 6 months. Various antiviral drugs have been used, including acyclovir, valaciclovir, intravenous ganciclovir, oral valganciclovir or intravenous (IV) ganciclovir, and valganciclovir. In preventive therapy, laboratory monitoring detects asymptomatic viral replication and antiviral therapy is initiated to prevent progression to clinical disease. For non severe CMV disease, oral valganciclovir (900 mg orally every 12 hr) or IV ganciclovir (5 mg/kg every 12hr) are recommended as first-line treatment. Renal function should be monitored frequently during treatment, with estimated or measured glomerular filtration rate. Optimal length of treatment should be achieved by monitoring weekly viral loads and treating until one or two consecutive negative samples are obtained, but not shorter than 2 weeks. Duration should reflect the likelihood of recurrent CMV infection. In cases of serious disease and in tissue-invasive disease without viremia, longer treatment periods with clinical monitoring of the specific disease manifestation are recommended. In cases of recurrent CMV disease, prophylaxis after retreatment may need to be prolonged. [44].

8. Transplantation

CMV seroprevalence is high in developing countries such as Brazil, so most of the patients and/or donors is CMV IgG positive. The techniques chosen for the laboratorial monitoring in our service after liver transplantation are antigenemia and Nested-PCR (N-PCR). These techniques detect the active viral replication and minimize the damage of the disease caused by CMV (see Section xy).

We diagnose active infection from one positive result by antigenemia, or two positive N-PCR findings over an interval equal or smaller than 30 days. As antigenemia can detect CMV a few days to one week before the appearance of the symptoms, the Ganciclovir is initiated after the detection of a positive cell even without clinical symptoms if the patient presents IgG negative and the donor presents IgG positive. Patients are monitored while in hospital and after discharge following a protocol: weekly from the first to the second month, fortnightly in the third-fourth months and monthly until six months. After this period the antigenemia or N-PCR is performed only if there is a suggestive clinical diagnosis of CMV infection. The assessment of antigenemia also provides an estimate of viral load that is useful in the differentiation of CMV disease from other complications. Thus we evaluate the efficacy of antiviral therapy and have capacity to detect drug resistance.

CMV is frequently detected in our patients after liver transplantation [24,25,30,31]. Detection of N-PCR and antigenemia are useful markers for active infection [30,31]. The rates of CMV found in our groups are similar to other services [24,25,30-32].

We also observed that symptomatic CMV infection occurs during the first three months after transplantation. We consider that this high incidence of symptomatic CMV infection is due to the high prevalence of the virus in Brazilian population. The mean time for initial detection CMV is around 29 days following transplantation (range: 0-99 days) [30].

In our service, CMV DNA diagnosed in pretransplantation graft biopsy specimens remained positive posttransplantation on graft biopsies. This common complication negatively influences liver transplantation outcomes and is a risk factor to develop acute cellular rejection episodes [67]. Ganciclovir prophylaxis for CMV is not performed at our institution unless the patient is preoperative negative IgG and the donor is CMV positive. Prophylaxis is performed only for herpes simplex type 1 with Acyclovir.

Another relevant issue at our service is opportunistic infections, which are often seen in patients at risk for CMV and have been recognized by our staff as a significant risk factor for graft failure and death [24]. Active CMV infection may increase the risk of bacterial, fungal, viral, and others, as well as post-transplant lymphoproliferative disease. [31] This includes co-infections by other viruses of the same family (HHV-6, HHV-7) [24,32].

The clinical impact of CMV-infected patients observed by our team [24] shows that it is extremely important to follow up these patients. These data have helped the medical staff making therapeutic strategies to minimize risks caused by this betaherpesvirus.

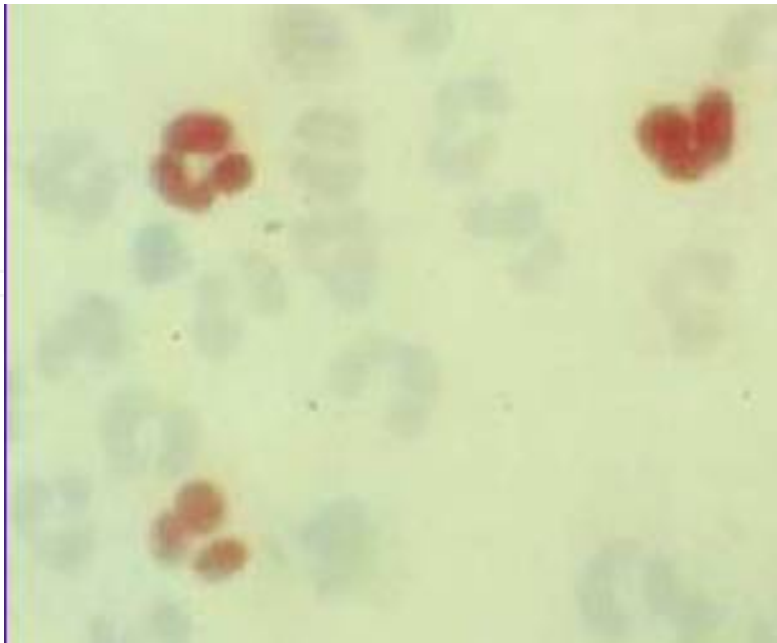


Figure 1. Nuclei of neutrophils stained in brown indicating positive pp65-antigenemia (counterstained with Harris’s hematoxylin). Mouse C10 and C11 monoclonal antibodies against pp65-matrix CMV antigen and rabbit anti-mouse Ig horseradish peroxidase conjugate. The reaction was revealed by hydrogen peroxide and amino-ethyl-carbazole²⁴

Virus	Synonym	Subfamily	Abbreviation
Human Herpesvirus-1	Herpes simplex-1		HSV-1/HHV-1
Human Herpesvirus -2	Herpes simplex-2		HSV-2/HHV-2
Human Herpesvirus -3	Varicella-zoster		VZV/HHV-3
Human Herpesvirus -4	Epstein-Barr		EBV/HSV-4
Human Herpesvirus -5	Cytomegalovirus		HCMV/CMV/HHV-5
Human Herpesvirus -6	None		HHV-6
Human Herpesvirus -7	None		HHV-7
Human Herpesvirus -8	None		KSHV/HHV-8

Table 1. Complete list of human herpesvirus

9. Conclusion

Few patients remain free of betaherpesvirus after liver transplantation. Active CMV infection is common especially in the first weeks after grafting. We believe it is important to continue monitoring CMV infection after transplantation, especially when the prevalence in the general population is high.

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