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MANAGEMENT OF HUMAN CYTOMEGALOVIRUS INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS: CONSENSUS OF THE “SOCIETA’ ITALIANA DI VIROLOGIA-SIV” AND THE “ASSOCIAZIONE MICROBIOLOGI CLINICI ITALIANI-AMCLI”*

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Introduction

Human cytomegalovirus (HCMV) infection is the most important viral complication and an important cause of morbidity and mortality in solid organ transplant recipients (SOTR). HCMV can cause systemic and organ infections, including the lungs, gastrointestinal tract, liver, kidney and central or peripheral nervous system. A prompt and appropriate diagnosis of HCMV infection and disease is mandatory in these patient populations, in order to timely intervene to prevent disease or to avoid incorrect management of the patient. A brief definition of the HCMV systemic syndrome and organ disease follows [1].

Systemic syndrome. Systemic HCMV syndrome is characterized by the following symptoms and signs: fever $>38^{\circ}\text{C}$ for at least 2 days, malaise, leukopenia, thrombocytopenia, elevation of hepatic transaminases >2 fold the normal upper limit (applicable to non-liver transplant recipients), presence of HCMV or its products in blood and exclusion of other possible causes.

End-organ disease. HCMV end-organ disease is defined by symptoms and signs of organ involvement (pulmonary, gastrointestinal, hepatic, neural or, less frequently, other organ sites) associated with either immunohistochemical or virological detection (viral DNA quantification or virus isolation) of HCMV in biopsy tissues (independently of virus presence in blood), in the absence of other possible causes of organ disease.

The following recommendations for management of HCMV infection are graded according to the United States Center for Disease Control as described in Table 1 [2].

Diagnosis and monitoring of (active) HCMV infection

Diagnostic tools. Active HCMV infection in blood can be diagnosed by detecting HCMV or its products in blood. Since the end of the 80s, different techniques have been developed for this purpose, allowing rapid and sensitive diagnosis of HCMV infection [3,4]: virus isolation (standard isolation with virus recovery, or rapid isolation by the shell-vial technique), detection of pp65 in peripheral blood leukocytes (pp65-antigenemia), detection and quantification of viral DNA in different blood compartments (DNAemia) and detection of immediate-early or late viral transcripts (IEmRNAemia or pp67mRNAemia).

Quantitative determination of viral load in blood has been shown to have a high prognostic value for the development of HCMV disease [5,6]. Among the different techniques, rapid virus isolation by the shell vial method, provides quantitative results highly correlating with actual viral replication and gives information on antiviral therapy effectiveness; however, it was found to lack sufficient sensitivity to reliably guide preemptive therapy [3,7].

The antigenemia assay has been widely used and has been currently adopted in many centres for diagnosis of HCMV infection and guidance of preemptive therapy [5]. Although the assay was shown to be suitable for standardization [8], interpretation of test results remains subjective and the assay is not automatable. In addition, due to the biological properties of pp65 (which is synthesized in excess in infected endothelial cells and passively transferred to blood leukocytes) [9], antigenemia quantification does not directly correlate with actual viral replication, providing in particular cases misleading information [10,11].

These limitations could be overcome by the introduction of molecular assays, among which viral DNA quantification by real-time PCR has proven to give highly reliable results, since it directly correlates with clinical symptoms and viral replication [12]. Viral DNA can be quantified in different blood compartments (leukocytes, plasma, or whole blood), but several studies have shown that whole blood is the specimen of choice for HCMV DNAemia quantification, since it allows determination of both cell-free and cell-associated virus [13,14]. However, since different systems (both those developed in-house or commercially available) are used in different transplantation centres, a standardized methodology is warranted, as well as periodical reference to external quality control panels by all laboratories involved in HCMV DNA quantification.

Viral organ localizations are diagnosed by examining organ biopsies (or, alternatively, local secretions, e.g. bronchoalveolar lavage fluid -BAL- for pulmonary infection) by either immunohistochemistry and/or virological assays (viral DNA quantification or virus isolation).

Serological assays for HCMV-specific IgG or IgM determination have no current use in the diagnosis and monitoring of HCMV infection in transplant recipients, unless diagnosis of primary vs reactivated infection is required.

Frequency of virological monitoring. Virological monitoring should be performed by HCMV DNAemia quantification on whole blood weekly during the first three months after

transplantation (or at least during the first 2 months, then every 2 weeks in the third month). When active HCMV infection is diagnosed (i.e. positive DNAemia) more frequent monitoring (2 tests/week) should be performed. This monitoring schedule has been shown in prospective studies [15-18] to timely detect patients at risk of developing HCMV infection, thus allowing the timely initiation of antiviral intervention. Beyond three months after transplantation, in order to avoid onset of late HCMV disease, monitoring should be performed: i) monthly (or at least in concomitance with routine medical visits; ii) in case of an increase in the immunosuppressive regimen due to rejection; and iii) on the basis of any clinical indication suggesting the presence of HCMV infection/disease. In case of an active HCMV infection, weekly or biweekly monitoring should be reinstated.

When organ localization is suspected, organ biopsy or local secretions should be examined, either in the presence or absence of HCMV or HCMV products in peripheral blood. In lung transplant recipients, HCMV-DNA monitoring in BAL in concomitance with routine bronchoscopy procedures for rejection surveillance is suggested [19,20].

Introduction of immunological monitoring. It is widely accepted that control of HCMV infection is conferred by reconstitution (or development) of the HCMV-specific cell-mediated immune response [21-24]. The severity of HCMV infection and the extent of organ involvement inversely correlate with the development or restoration of an efficient CD4⁺ and CD8⁺ T-cell immune response, while the absence of T-cell immunity is consistently associated with recurrent episodes of reactivated HCMV infection. Several, yet not standardised, techniques are utilized to monitor HCMV-specific CD4⁺ and CD8⁺ T-cell immune responses [25-27].

However, from the clinical standpoint, it appears reasonable to assume that simultaneous immunological and virologic follow-up of individual patients may improve management of HCMV infections in transplanted patients, thereby avoiding strict virologic monitoring/treatment of patients with apparently efficient T-cell immunity. This should be verified in future prospective studies. Episodes of steroid-treated organ rejection indicate, in the presence of a relapse of HCMV infection, the need for re-initiation of virologic monitoring for at least 2-4 weeks or until viral disappearance.

Recommendations

- Quantitative determination of viral load in blood is indicated since it has a high prognostic value for the development of HCMV disease (A I).
- HCMV DNA quantification on whole blood is the elective assay for monitoring viral load, since it directly correlates with viral replication and clinical symptoms; however, periodical reference to external quality control panels is mandatory (A I).
- For organ localization diagnosis, an organ biopsy (or, alternatively, local secretions e.g. bronchoalveolar lavage fluid for pulmonary infection) should be examined (A III).
- DNAemia quantification on whole blood should be performed weekly during the first three months after transplantation. When active HCMV infection is diagnosed, more frequent monitoring (2 tests/week) should be performed (A I).
- Beyond three months after transplantation, in order to avoid onset of late HCMV disease, monitoring should be performed monthly (or at least in concomitance with routine medical visits) in case of an increase in the immunosuppressive regimen for rejection, and on the basis of symptoms and signs suggesting HCMV infection/disease [1] (B II).
- The role of HCMV-specific immune response monitoring in transplanted patient management should be verified in future prospective studies (research need).

Prevention of HCMV disease

Preemptive therapy vs prophylaxis. Due to the high morbidity and mortality rate associated with HCMV disease in the transplantation setting in the absence of adequate intervention, the optimal management strategy for the control of HCMV infection is the prevention of overt disease. The two main approaches for prevention of HCMV disease are antiviral prophylaxis and preemptive therapy using the currently available anti-HCMV compounds [Ganciclovir (GCV), Valganciclovir (VGCV), Foscarnet (PFA) and Cidofovir (CDV)]. Even if both strategies are able to reduce the incidence and severity of HCMV disease, the elective approach appears to be preemptive strategy, while prophylaxis should be adopted only in transplantation centres with no facilities for virological monitoring. The major advantage of antiviral prophylaxis (that is currently based on the administration of antiviral drug for 100 days after transplantation) relies on its easy use. However,

possible drawbacks of universal prophylaxis are that prolonged administration of antiviral drugs exposes patients to the toxic effects of antiviral compounds and, especially in the presence of suboptimal drug levels, could induce the emergence of drug-resistant HCMV strains [28,29], even though thus far no study has demonstrated a significantly higher rate of antiviral resistant strains in patients receiving prophylaxis with respect to preemptive therapy. In addition, antiviral prophylaxis does not completely prevent the occurrence of HCMV infection and disease, which can affect patients after prophylaxis cessation. Indeed, late HCMV disease is an important clinical problem in transplant recipients who receive antiviral prophylaxis. It involves about 5-18% of patients and is strongly and independently associated with mortality [30]. In addition, the potential interfering role of prophylaxis with the development of HCMV-specific T-cell immune response is still debated.

On the other hand, preemptive therapy consists of the administration of antiviral drugs when viral load in blood reaches a level predictive of HCMV disease development, but before the onset of clinical symptoms. The major advantage of preemptive therapy with respect to prophylaxis is that only a minor proportion of patients is treated for a shorter period of time, since not all patients undergoing HCMV infection in the post-transplant period are at risk of developing HCMV disease and, thus, require antiviral therapy. However, preemptive therapy requires continuous virological monitoring to be efficacious in preventing HCMV disease. Nonetheless, preemptive therapy represents savings in terms of drug toxicity and patient management costs [31]. In addition, some authors have not reported better efficacy of prophylaxis either in high risk patients or in the prevention of the hypothesized indirect effects (such as exposure to higher risk of graft failure or bacterial and fungal infections) of subclinical HCMV infection [32-36].

Prevention of HCMV disease in SOTR. The recommended preventive strategy for SOTR is preemptive therapy with i.v. GCV (5mg/kg/bid) administered until the disappearance of virus from blood. This strategy has been shown in different prospective studies to almost completely prevent HCMV disease [17,18,32,37]. Recent evidence for the efficacy of VGCV p.o. (900mg/bid) was provided by some authors [36,38-40]. However its efficacy still needs to be validated in the pediatric population. There are no controlled trials on the use of PFA and CDV in preemptive treatment of HCMV infection in SOTR. However, although there is proof of efficacy of these drugs in treating HCMV disease, they are recommended as a second line choice due to their high toxicity

profile. It has been observed that the combined administration of i.v. PFA and i.v. GCV at half doses does not lead to better control of HCMV infection than full dose i.v. GCV alone [41].

A cost-effective HCMV DNAemia cutoff of 300,000 copies/ml blood for safe initiation of preemptive therapy was recently validated in a large bicentric prospective trial involving 200 patients receiving an heart, lung, liver or kidney transplantation. GCV was administered i.v. in patients reaching the DNAemia cutoff until confirmed disappearance of virus from blood [18]. In addition, the same study showed that both primary and reactivated infections can be safely managed using the same cutoff. In order to export this cutoff to different centres, standardization of the different commercial and in-house developed methods for DNAemia determination is being performed by a committee of the two societies proposing the present guidelines (manuscript in preparation). The multicentric evaluation performed showed an acceptable inter-laboratory range of variation ($\pm 0.5 \log_{10}$, or less for high HCMV DNA quantity). Thus, taking into account the observed inter-laboratory variability of the assays, the optimal cutoff for pre-emptive therapy to be exported among different centres is 300,000 ($5.5 \log_{10}$) $\pm 0.5 \log_{10}$ copies/ml. In other words, it can be safely recommended to start preemptive therapy in the presence of $\geq 100,000$ ($5 \log_{10}$) copies/ml blood. In addition, due to the reproducibility of HCMV-DNA quantification among different laboratories, outpatients living far from their transplantation centre can rely on other laboratories for virologic monitoring. In this respect, a network of Reference Laboratories (subject to periodical external quality control) should be created and acknowledged by the National Transplant Centre.

Since there is the possibility that in some cases HCMV organ localization can occur in the presence of low or absent viremia, it has been suggested by different authors to monitor HCMV DNA in BAL of lung transplant recipients concomitantly with routine sampling for rejection surveillance, and to start preemptive therapy in the presence of high DNA levels in BAL. A tentative cutoff of $5 \log_{10}$ (100,000) HCMV DNA copies/ml BAL has been proposed [19,20] and should be investigated in prospective controlled trials. In addition, the best prevention strategy in particular types of transplantation (i.e transplant in HIV-seropositive subjects, multivisceral transplant) remains to be investigated.

Recommendations

- The elective approach for prevention of HCMV disease is the preemptive (A II).
- A network of Reference Laboratories should be created that outpatients can be referred to when living far from their transplantation centre (AIII).
- The recommended preventive strategy for SOTR is preemptive therapy with iv GCV (5mg/kg/bid). Therapy should be initiated after reaching a DNAemia level over 5 log₁₀ (100,000) copies/ml blood and continued until DNAemia becomes negative. The same cutoff can be used for both primary and recurrent infections (A I).
- VGCV p.o. (900 mg/bid) can be used as an alternative to GCV i.v. for preemptive therapy of SOTR (A II); effectiveness of VGCV p.o. should be assessed in the pediatric population (research need).
- Due to the emergence of GCV toxicity or, although rarely, GCV resistance, PFA and CDV can be used as a second choice for preemptive therapy of SOTR (B II).
- Monitoring of HCMV DNA load in BAL of lung transplant recipients to guide preemptive therapy is suggested (B III); however, the appropriate cutoff for initiation of preemptive therapy needs to be determined (research need).
- The optimal prevention strategies should be determined in special patient populations, such as multivisceral transplant recipients and HIV-seropositive SOTR (research need).

Treatment of HCMV disease.

Preemptive therapy, if guided by appropriate virological monitoring, is virtually able to prevent any case of overt HCMV disease. However, the preventive strategy may fail in case of organ involvement not associated with the presence of virus or viral products in blood. In case of established HCMV disease, i.v. administration of GCV (5mg/kg/bid) or PFA (90mg/kg/bid) is the first choice treatment. Therapy should be protracted for 2-4 weeks or until disappearance of virus from the involved organ or blood. The established therapy in case of HCMV pneumonia is the combination of i.v. GCV and high dose immunoglobulin. However, it is still controversial whether administration of immunoglobulin or even HCMV hyperimmune globulin can improve outcome. No organ involvement other than lungs requires use of immunoglobulin, unless agammaglobulinemia is documented. VGCV p.o. (900mg/bid) appears to be a valid alternative to GCV also for treatment of HCMV disease [42-45]. However, its efficacy remains to be demonstrated in the paediatric population and in case of altered intestinal absorption. CDV can be used as a second line choice. Finally, the utility and effectiveness of adoptive immunotherapy [46] both for the prevention and treatment of HCMV disease in patients who are not able to spontaneously develop immune control of HCMV infection should be evaluated in future prospective studies.

Pharmacokinetics and drug resistance.

The most clearly defined variable affecting pharmacokinetic parameters is renal impairment. GCV clearance correlates with creatinine clearance, providing a basis for dosage adjustment in patients with renal impairment. In the presence of altered renal function, the dose of GCV and VGCV, for both prevention and treatment of HCMV disease, may be reassessed also on the basis of plasma drug concentration, in order to prevent drug accumulation and risk of toxic effects due to higher drug exposure.

Although GCV pharmacokinetic parameters have been defined, high inter- and intraindividual variability was observed during clinical use. The role of therapeutic monitoring has not been clearly addressed and a therapeutic range for plasma GCV concentrations has not been clearly defined.

The available data on the antiviral activity of GCV against HCMV report that the *in vitro* concentration required to achieve 50% viral inhibition (IC_{50}) ranges from 0.75 to 1.5 $\mu\text{g/ml}$ [47], but can reach up to 2.0 $\mu\text{g/ml}$ [48]. GCV is virustatic with the ID_{50} for most clinical HCMV isolates ranging from 0.2 to 1.6 mcg/mL [49]. These *in vitro* susceptibilities have been used as the basis for guiding target drug concentrations in pharmacokinetic studies.

Several studies in solid organ transplant recipients receiving GCV for HCMV prophylaxis or treatment report a wide range of plasma drug concentrations, with trough concentrations (C_{trough}) ranging from 0.06 to 11.7 $\mu\text{g/ml}$ and peak concentrations (C_{max} : 15 min. after i.v. and 3h after oral administration) ranging from 0.96 to 22.1 $\mu\text{g/ml}$ [50]. On the basis of different pharmacokinetic studies and guidelines, it can be concluded that C_{trough} should be around the reported value of 1.0 $\mu\text{g/ml}$. However, it can be speculated that C_{trough} higher than 2.0 mcg/mL might be even more effective, especially for patients at high risk [51]. A $C_{\text{trough}} > 5.0 \mu\text{g/ml}$ and a $C_{\text{max}} > 20 \mu\text{g/ml}$ may be close to the onset of toxic effects.

In case of DNAemia (or viremia) increase during treatment, an investigation for detection of a drug-resistant HCMV strain should be taken into consideration. It should be emphasized, however, that a rise in antigenemia level alone during the first 10-15 days of treatment (not accompanied by a concomitant rise in DNAemia and viremia levels) does not indicate therapy failure but, rather in primary infections of SOTR, is due to an excess pp65 synthesis during viral replication. [10,11]. When a rise in DNAemia or viremia occurs during antiviral treatment, the emergence of drug resistant HCMV strains must be confirmed by drug resistance assays. Detection of mutations in the HCMV UL97 or UL54 genes (genotypic drug resistance assay), allows a rapid diagnosis of drug resistance and provides helpful informations for selection of alternative antiviral treatments [52]. A lack of antiviral treatment effectiveness may also occur in the absence of mutations in the viral genome. In such case, an evaluation of the actual plasma drug concentration and a re-evaluation of immunosuppression is required. Empirical treatment shift should always be avoided in order to prevent emergence of multidrug resistant strains.

Recommendations

- In case of established HCMV disease, i.v. administration of GCV (5mg/kg/bid) or PFA (90mg/kg/bid) for 2-4 weeks or until disappearance of virus from blood or from the involved organ is the first choice treatment (A II). It is not clear if the concomitant administration of i.v. immunoglobulin for treatment of HCMV pneumonia adds any benefit (C III).
- VGCV p.o. (900mg/bid) appears to be a valid alternative to GCV also for the treatment of HCMV disease (AII). Its efficacy remains to be documented in the paediatric population and in case of altered intestinal absorption (research need).
- CDV may be considered as a second line treatment (B II).
- In case of renal function impairment, GCV or VGCV dosage should be reassessed on the basis of plasma drug concentration (A III).
- Plasma samples (C_{trough}) must be obtained after at least 2 days (*depending on renal function: 4 days for patients with severe renal impairment*) from the start of the dosing regimen or from a dosage regimen modification. C_{trough} should be maintained around the value of 1.0 µg/ml. In patients at high risk, trough levels higher than 2.0 µg/ml might be even more effective. $C_{\text{trough}} > 5.0$ µg/ml may be closely related to the onset of toxic effects: in case of toxicity consider dose reduction (AIII).
- In case of a DNAemia increase during treatment, the possible selection of a drug-resistant strain should be verified by a phenotypic/genotypic assay. The antiviral drug should be changed only in case of detection of a drug-resistant strain. When absence of drug resistant strains has been documented, the plasma antiviral drug concentration or immunosuppression should be re-evaluated (A III).
- The effectiveness of adoptive immunotherapy for either prevention or treatment should be investigated (research needed).

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Table 1 Grading recommendations according to the United States Center for Disease Control (adapted from Gross et al., 1994)

Strength of recommendations	
A	Strong evidence for efficacy and substantial clinical benefit. Strongly recommended.
B	Strong or moderate evidence for efficacy, but only limited clinical benefit. Generally recommended.
C	Insufficient evidence for efficacy; or efficacy does not outweigh possible adverse consequences or costs of chemoprophylaxis or alternative approaches.
D	Moderate evidence against efficacy or of adverse outcome. Generally not recommended.
E	Strong evidence against efficacy or of adverse outcome. Never recommended.

Quality of evidence supporting recommendation.	
I	Consistent evidence from controlled clinical trial. Evidence from at least one well-designed randomized trial and, in case of laboratory studies, consistent evidence from comparative studies.
II	Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytical studies (preferably from more than one centre), or from multiple time-series studies or dramatic evidence from uncontrolled experiments.
III	Evidence from opinion of respected authorities based on clinical experience, descriptive studies or reports from expert committees.
