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MANAGEMENT OF HUMAN CYTOMEGALOVIRUS INFECTION IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS: GUIDELINES OF THE "SOCIETA' ITALIANA DI VIROLOGIA-SIV"

Introduction

Human cytomegalovirus (HCMV) infection is the most important viral complication and an important cause of morbidity and mortality in haematopoietic stem cell transplant recipients (HSCTR). HCMV can cause systemic and organ infections, including the lungs, gastrointestinal tract, liver, kidney and central or peripheral nervous system, and the disease can develop both early (within 100 days) and late after transplantation. A prompt and appropriate diagnosis of HCMV infection and disease is mandatory in this patient population, in order to timely intervene to prevent disease or to avoid incorrect management of the patient. A brief definition of HCMV systemic syndrome and organ disease follows [1].

Systemic syndrome. Systemic HCMV syndrome is characterized by the following symptoms and signs: fever >38°C for at least 2 days, malaise, leukopenia, thrompocytopenia, elevation of hepatic transaminases >2 fold the upper normal 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. Gastrointestinal disease and pneumonia are the most common manifestations in HSCT recipients.

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

Diagnosis 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 in both HSCTR and solid organ transplant recipients [5-7]. Among the different techniques, rapid virus isolation by the shell vial method, provides quantitative results that highly correlate with actual viral replication and gives information on antiviral therapy effectiveness; however, it was found to lack sensitivity for guiding preemptive therapy [3,8].

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 [6,9]. Although the assay was shown to be suitable for standardization [10], interpretation of test results remains subjective and the assay is not automatable, in view of the need to manage an increasingly high number of samples per day. In addition, due to the biological properties of pp65 (which is synthesized in excess in infected endothelial cells and passively transferred to blood leukocytes) [11], antigenemia quantification does not directly correlate with actual viral replication, providing in particular cases misleading information [12-13].

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. 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 [14-17]. 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 [18].

Viral organ localization is 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 are not useful in the diagnosis and monitoring of HCMV infection in HSCTR except for defining the risk level for HCMV infection development when performed in the donor and the recipient before transplantation. However, it should be noted that low levels of IgG specific for HCMV could be detected in candidate patients for transplantation if they received blood products or immunoglobulin administration, even if they had not previously encountered the virus [19]

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, standardization of the different methods 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).

Prevention of HCMV disease

Preemptive therapy vs prophylaxis. Due to the high mortality rate associated with HCMV disease in the transplantation setting, 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 approaches are able to reduce the incidence and severity of HCMV disease, the approach of choice appears to be the preemptive strategy. The major advantage of antiviral prophylaxis, that is currently based on the administration of GCV from engraftment till day +100 [20-22], relies on its easy use**.** However, while GCV prophylaxis has been associated with a reduction for the risk of HCMV disease as compared to placebo, no improvement has been observed in overall survival. Possible drawbacks of universal prophylaxis are that prolonged administration of antiviral drugs expose patients to the toxic effects of antiviral compounds, such as neutropenia induced by GCV given at engraftment, leading to more invasive bacterial and fungal infections [20,22-23]. Moreover, especially in the presence of suboptimal drug levels, sustained administration of antivirals could induce emergence of drug-resistant HCMV strains, 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. Furthermore, its potential interference with a delay in HCMV-specific T-cell reconstitution is still debated [24-25].

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, 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, while using a prophylactic approach, a substantial number of patients not at risk for disease (about 60-70%) will receive an unnecessary but potentially toxic antiviral drug. However, preemptive therapy requires continuous virological monitoring to be efficacious in preventing HCMV disease, and depends on adherence to

the testing schedule. Disease may occur in case of missed surveillance, and, in the absence of virus in blood, in rare cases of organ localization (especially gastro-intestinal) [26].

Prevention of HCMV disease in HSCTR. The recommended strategy for prevention of HCMV disease in HSCTR is preemptive therapy with i.v. GCV (5mg/kg/bid) or i.v. PFA (90mg/kg/bid) until disappearance of virus from blood [22,27-33]. A randomised prospective trial showed that GCV and PFA are equally effective, while serum electrolyte abnormalities were more common in the PFA group and neutropenia in the GCV group [33]. The choice should depend on the evaluation of the toxicity risk. The combined administration of i.v. PFA and i.v. GCV at half doses does not improve control of HCMV infection with respect to administration of i.v. GCV alone at full dosage [34]. Thus far, no controlled trial on CDV has been conducted, and this antiviral drug should only be used as a second line choice due to its toxicity profile. The efficacy of VGCV p.o. is still under evaluation, especially in the pediatric population and in case of altered intestinal absorption (such as that occurring in patients with GvHD). However, there is recent positive evidence of its pharmacokinetic and clinical utility in HSCTR [35-39]. Preemptive therapy was generally initiated after first (confirmed) HCMV detection in blood, whichever test (antigenemia or DNAemia) was used. However, two recent trials performed in both pediatric and adult HSCT patients [31-32] and an observational study [40] showed that adoption of a cutoff level of 4 $\log_{10}$ (10,000) HCMV DNA copies/ml blood to start preemptive therapy (until confirmed DNAemia negativity), is safe and more cost-effective (significant reduction in number of patients receiving preemptive therapy) than starting treatment upon the first positive result.

Apart from the above considerations, the administration of standard or hyperimmune globulin has no or at best little use in the prevention of HCMV infection or disease [41-43]. Moreover, it remains to be determined which is the best prevention strategy in special transplantation settings such as HSCT in HIV-seropositive patients.

Frequency of virological monitoring. The effectiveness of preemptive therapy in preventing disease relies on virological monitoring. This should be performed weekly during the first three months after transplantation, by HCMV DNAemia quantification on whole blood. When an active HCMV infection is diagnosed (i.e. positive DNAemia) more frequent monitoring (2 tests/week) should be performed until virus clearance from blood. This monitoring schedule has been shown in

prospective studies [22,29-33] to be able to detect patients at risk of developing HCMV infection, thus allowing timely initiation of antiviral therapy. Beyond three months after transplantation, in order to avoid onset of late HCMV disease, monitoring should be performed: i) monthly (or at least concomitantly with routine medical visits) until 12 months after transplantation; ii) in case of an increase in the immunosuppressive regimen due to GvHD; and iii) on the basis of any clinical indication suggesting the presence of HCMV infection/disease. In case of diagnosis of active HCMV infection, weekly or biweekly monitoring should be reinstated.

When an 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.

Introduction of immunological monitoring. For several years it has been known that control of HCMV infection is conferred by reconstitution (or development) of the HCMV-specific cell-mediated immune response [24,44-45]. In particular, 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, whereas 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 [46-51].

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 infection in transplanted patients. This strategy (see Figure 1) would allow tailored surveillance and prevention strategies on the basis of the patient's immune reconstitution, thereby avoiding strict virological monitoring/treatment of patients with efficient T-cell immunity. On the contrary, patients lacking HCMV-specific immune reconstitution, or receiving steroid treatment for GvHD, should undergo sustained virologic monitoring in order to prevent late disease [52-53]. This approach, although already proposed for control of HCMV late disease after the first three months post transplant [52], should be verified in future prospective trials.

Recommendations

- The elective approach for prevention of HCMV disease is the preemptive strategy, while prophylaxis should be adopted only in transplantation centres with no facilities for virological monitoring (A II).
- The recommended preventive strategy for HSCTR is preemptive therapy with i.v. GCV (5mg/kg/bid) or i.v. PFA (90mg/kg/bid). Some studies indicate as a cost-effective cutoff for initiating pre-emptive therapy a DNAemia level of 4 $\log_{10}$ (10,000) copies/ml blood. Therapy should be protracted until DNAemia becomes negative (A I).
- VGCV p.o. (900mg/bid) can be used as an alternative to GCV in HSCTR (AII). Its efficacy should be verified in the pediatric population and in case of altered intestinal absorption (research need).
- CDV could be used as a second line choice for preemptive therapy of HSCTR (B II).
- The optimal prevention strategies should be determined in special patient populations, such as HIV-seropositive HSCTR (research need).
- 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 post-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 GvHD, and on the basis of any clinical indication (B II).
- The role of HCMV-specific immune response monitoring in transplanted patient management should be verified in future prospective studies (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 in the absence 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 [54-56]. No organ involvement other than lung requires use of immunoglobulin. VGCV p.o. (900mg/bid) was shown to be a valid alternative to GCV also for treatment of HCMV disease in organ transplant recipients [57-60]. However, its efficacy remains to be demonstrated in HSCTR. CDV can be used as a second line choice. Finally, the utility and effectiveness of adoptive immunotherapy [61] both for the prevention and treatment of HCMV disease in patients unable 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, thus 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 are defined, high inter- and intraindividual variability have been observed during clinical use. The role of therapeutic monitoring has not been clearly addressed and a therapeutic range for plasma GCV concentration has not yet been clearly defined.

The available data on the antiviral activity of GCV against HCMV show that the *in vitro* concentration required to achieve 50% viral inhibition (IC_{50}) ranges from 0.75 to 1.5 μg/ml [62], but can reach up to 2.0 μg/ml [63]. GCV is virostatic with an ID_{50} for most clinical HCMV isolates ranging from 0.2 to 1.6 μg/ml [64]. 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 μg/ml and peak concentrations (C_{max}: 15 min. after i.v. and 3h after oral administration) ranging from 0.96 to 22.1 μg/ml [65]. 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 μg/ml. However, it can be speculated that a C_{trough} higher than 2.0 mcg/mL might be even more effective, especially for patients at high risk [66]. A C_{trough} > 5.0 μg/ml and a C_{max} > 20 μg/ml may be associated with the onset of toxic effects.

In case of DNAemia (or viremia) increase during treatment, an investigation for HCMV drug-resistance should be undertaken. 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, as in primary infections of SOTR, is due to excess pp65 synthesis during viral replication. [12,13]. 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 indications for the selection of alternative antiviral treatments [67]. A lack of antiviral treatment effectiveness may also occur in the absence of mutations in the viral genome. In such cases, an evaluation of the actual plasma drug concentration and a re-evaluation of the immunosuppressive regimen is required. Empirical treatment shifts 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 whether 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 for the treatment of HCMV disease in organ transplant recipients (AII). Its efficacy remains to be documented in HSCTR (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).
- In case of a DNAemia increase during treatment, a 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. Otherwise, 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 Recommendations and grading according to the United States Centers 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(s). 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.

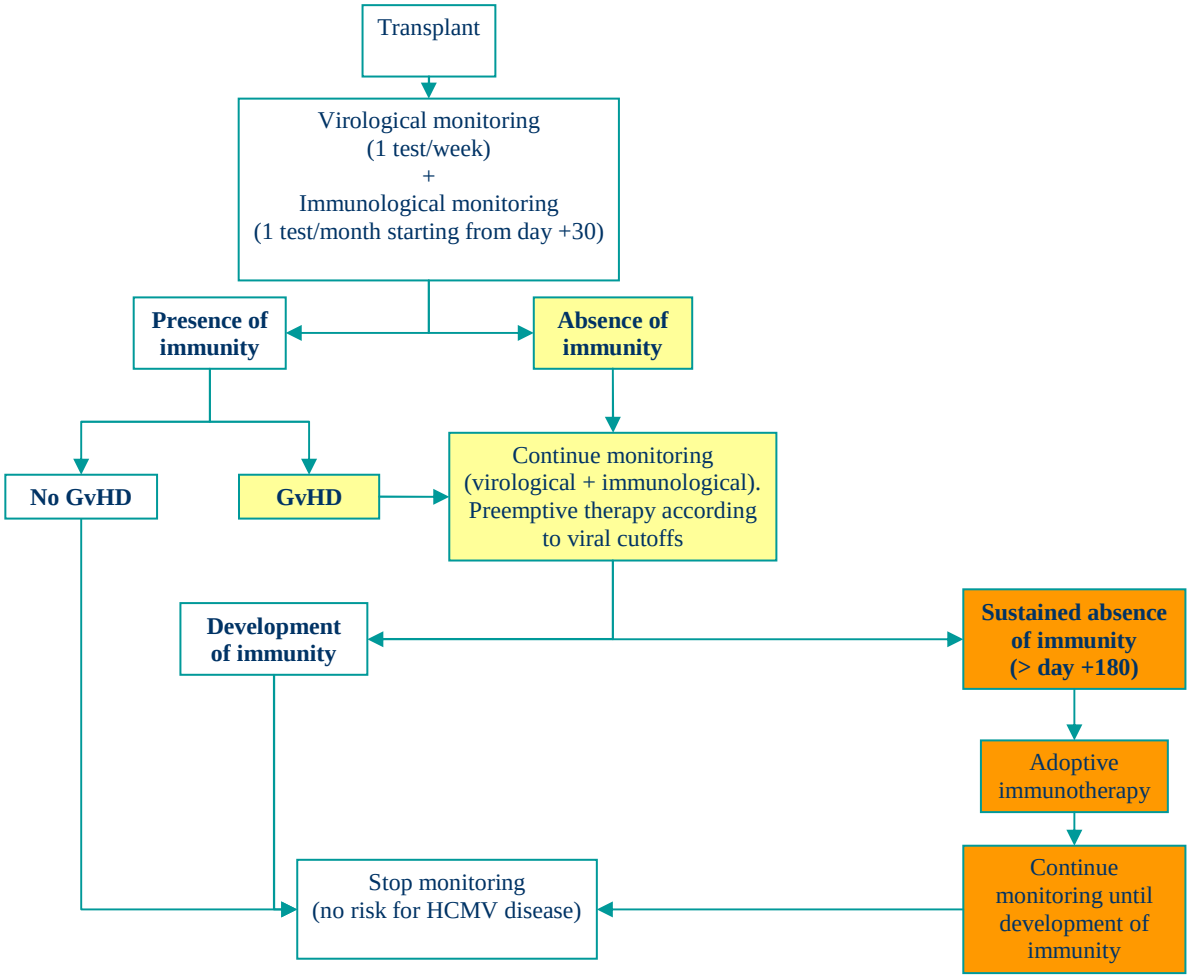

Figure 1. Combination of virologic and immunologic monitoring. After transplantation, patients undergo both virologic and immunologic monitoring to detect HCMV infection and development (or reconstitution) of HCMV-specific immune response. When immune responses to HCMV are detected, and in the absence of GvHD, patients are no longer considered at risk for HCMV disease, and HCMV surveillance can be interrupted (blank boxes). Otherwise (prior to achieving a complete T-cell response and when receiving steroid treatment for GvHD), HCMV monitoring should be continued or reinstated (yellow boxes). Patients with sustained lack of specific immunity are candidates for adoptive immunotherapy (orange boxes).