Management of cytomegalovirus infection in haemopoietic stem cell transplantation

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Keywords: stem cell, transplantation, bone marrow, peripheral blood stem cells, cytomegalovirus.

List of Recommendations

- Cytomegalovirus (CMV) infection and CMV disease should be diagnosed according to established, internationally accepted, standardized criteria (Grade 1C).
- Risk-adapted patient assessment should inform clinical management (Grade 1B).
- All potential haemopoietic stem cell transplantation (HSCT) recipients should be tested for the presence of CMV IgG antibody at diagnosis (Grade 1C).
- Once optimum human leucocyte antigen (HLA) matching has been performed, a CMV IgG-negative donor should be chosen for a CMV IgG-negative recipient and a CMV IgG-positive donor should be chosen for a CMV IgG-positive recipient when possible (Grade 1A).
- Donors or recipients who are initially found to be CMV IgG-negative should be retested pre-transplant to exclude primary CMV infection (Grade 1C).
- Apparent CMV seroconversion in potential allograft recipients who have received unscreened blood products should be actively investigated to exclude passive acquisition of antibody (Grade 1C).
- Any CMV IgG-negative HSCT recipient transplanted from a CMV IgG-negative donor who develops CMV infection post-transplant must be reported to the Serious Hazards of Transfusion (SHOT) scheme (Grade 1C).
- Primary prophylaxis with ganciclovir is not generally recommended as toxicity outweighs efficacy in HSCT patients (Grade 1B).
- Primary prophylaxis with aciclovir or valaciclovir can be deployed but only in conjunction with appropriate monitoring of CMV in blood (Grade 1B).
- Valaciclovir or valganciclovir are valid treatment options for secondary prophylaxis with appropriate monitoring of CMV in blood (Grade 1C).
- Intravenous immunoglobulin is not recommended for prophylaxis of CMV infection (Grade 1C).
- Real time quantitative polymerase chain reaction (PCR) is the preferred choice for monitoring CMV DNA levels in HSCT patients (Grade 1B).
- All diagnostic laboratories should deploy the CMV international standard to allow viral loads to be compared between centres (Grade 1C).
- Monitoring of CMV load should be undertaken at least weekly for the first 3 months post-HSCT (Grade 2C).
- CMV viral load monitoring should continue for 6-12 months if the patient has chronic graft-versus-host disease (GvHD) or prolonged T-cell immunodeficiency (Grade 1B).
- Each transplant centre should have a risk-adapted policy detailing threshold values for treatment of CMV infection, taking into account patient factors and PCR methodology (Grade 2C).
- Ganciclovir is recommended as first line pre-emptive therapy for CMV in HSCT patients (Grade 1A).
- Oral valganciclovir is a valid alternative when gastrointestinal absorption is normal or only minimally impaired (Grade 1A).
- Foscarnet is recommended as an alternative first-line agent if neutropenia is present or for ganciclovir treatment failure (Grade 1A).

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• Pre-emptive therapy with cidofovir can be considered as third-line in patients unresponsive to, or intolerant of, both a ganciclovir preparation and foscarnet (Grade 2B).

• In patients in whom CMV DNA loads in blood increase by 1 log_{10} over 2 weeks of pre-emptive therapy with a first line drug, an alternative agent and drug resistance profiling should be considered (Grade 2C).

• Drug resistance should start to be suspected if CMV loads in the blood fail to respond after 14 d of therapy, especially in non-lymphopenic or multiply pre-treated patients (Grade 2C).

• Sequence analysis of the UL97 and UL54 genes is the preferred option for resistance screening for currently available drugs (grade 1B).

• A multidisciplinary approach to management of CMV disease is required (Grade 1C).

• De novo CMV disease should be treated with ganciclovir or foscarnet monotherapy and intravenous immunoglobulin (Grade 1B).

• CMV disease that develops while on pre-emptive therapy or is clinically progressive requires drug resistance testing, increased drug doses and/or combination therapy (Grade 1B).

• Reduction in immunosuppression, especially reduction in corticosteroid dose, is strongly recommended when possible (Grade 1B).

Scope

These evidence-based guidelines expand and adapt previous guidance (Tomblyn et al, 2009; Andrews et al, 2011). While specifically focusing on alloengenic haemopoietic stem cell transplantation (HSCT), they are relevant to other areas of haematological oncology where there is an increased risk of cytomegalovirus (CMV) infection, such as haematological cancers where intense anti-T-cell therapy has been deployed (O’Brien et al, 2006).

Methodology

The production of these guidelines involved the following steps:

• Establishment of a working group comprising experts in the field of alloengenic transplantation and clinical virology followed by literature review to 1 May 2012 including Medline, Pubmed and Cochrane reviews databases.

• Development of key recommendations based on randomized, controlled trial evidence. Due to the paucity of randomized studies, some recommendations are based on literature review and a consensus of expert opinion.

• The GRADE nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations (http://www.bchsguidelines.com/BCSH_PROCESS/EVIDENCE_LEVELS_ANDGRADES_OFRECOMMENDATION/43_GRADE.html).

• Initial review of manuscript, performed by the UK Clinical virology Network, British Society of Blood and Marrow Transplantation (BSBMT) executive committee and the British Committee for Standards in Haematology (BCSH) Haem-Onc Task Force.

• Final Review by sounding boards of the British Society for Haematology (BSH) and British Society of Blood and Marrow Transplantation (BSBMT).

Background

Clinical manifestations of CMV

CMV is a herpes virus. Primary infection is followed by lifelong latency. Clinical manifestations vary widely and should be diagnosed according to established, internationally accepted, standardized criteria (Ljungman et al, 2002a). CMV infection is diagnosed when CMV is detected, most commonly in the blood, using sensitive screening tools. It is termed primary CMV infection when infection occurs in a CMV IgG-negative patient and CMV reactivation when the patient, or donor, is known to be CMV antibody positive. In uncomplicated CMV infection, organ-specific signs and symptoms are absent, although non-specific symptoms, such as fever and malaise, may occur (Ljungman et al, 2002a, 2011). The diagnosis of CMV disease requires the presence of symptoms and signs compatible with end organ damage, together with the detection of CMV by a validated method in an appropriate clinical specimen (Ljungman et al, 2002a). If left untreated, asymptomatic CMV infection can progress to CMV disease, most commonly affecting the lung, gastrointestinal tract, eye, liver or central nervous system (CNS). CMV pneumonia is the most serious complication with a > 50% mortality (Ljungman, 1995). In addition to specific organ damage, CMV also has profound, poorly characterized, indirect immunosuppressive effects, leading to an increased incidence of fungal and bacterial infection (Nichols et al, 2002; Hakki et al, 2003; Blanquer et al, 2011) as well as higher rates of both acute and chronic graft-versus-host disease (GvHD) (Soderberg et al, 1996; Larsson et al, 2004; Wang et al, 2008). While some recent reports have suggested reduced relapse rates in selected patients who reactivate CMV (Behrendt et al, 2009; Elmaagacli et al, 2011), this finding remains controversial and should not hinder early aggressive treatment of CMV infection.

Risk factors for CMV infection

Post HSCT, most patients will reactivate latent virus rather than acquire primary infection. Mechanisms of latency are not completely understood but prevention of reactivation appears to be primarily dependent upon a persistent, robust,
T-cell-mediated, immune response (Sylweater et al, 2005; Ljungman et al, 2011). Not surprisingly, the intensity of immunosuppression and the degree of T-cell depletion in transplant protocols both critically affect the risk of reactivation (van Burik et al, 2007). Recipients of transplants from unrelated or human leucocyte antigen (HLA)-mismatched donors, where immuno-suppression is increased and GVHD more likely, are particularly likely to reactivate CMV and these patients have a survival disadvantage. The deployment of T-cell depleting agents, such as alemtuzumab, or antithymocyte globulin (ATG) or increased/prolonged courses of immunosuppression to treat GVHD significantly increases the risk of CMV infection (Schmidt-Hieber et al, 2010). These risk factors can also increase the risk of persistent, recurrent or late onset disease (Büyck et al, 2010), usually associated with impaired immune reconstitution (Hakkki et al, 2003; Zhou et al, 2009). Conversely, the reconstitution of specific anti-CMV cytotoxic T-lymphocytes (CTL) has been shown to be protective (Lamba et al, 2005). CMV disease is as much of a problem following non-myeloablative transplantation as it is in the myeloablative setting. Conflicting data have been reported regarding the relative risk of bone marrow (BM) compared to peripheral blood stem cells (PBSC) as a stem cell source (Nakamae et al, 2009; George et al, 2010; Pinana et al, 2010; Guerrero et al, 2012). Published incidence of CMV reactivation and CMV disease after umbilical cord blood transplantation (UCBT) varies significantly. UCBT recipients have been reported to be more susceptible to late complications of CMV, although this is not a universal finding and the relative role of T cell depletion in UCBT conditioning protocols requires further investigation (Boeckh et al, 2004; Takami et al, 2005; Walker et al, 2007; Beck et al, 2010; Brown et al, 2010; Sauter et al, 2011; Chiesa et al, 2012; Mikulska et al, 2012).

Recommendations

- CMV infection and CMV disease should be diagnosed according to established, internationally accepted, standardized criteria (Grade 1C).
- Risk-adapted patient assessment should inform clinical management (Grade 1B).

Impact of host and donor CMV serostatus

CMV screening

CMV screening should be performed using commercially available CMV IgG assays. There is no gold standard assay and it is important that laboratories running these tests participate in external and internal quality assurance schemes.

CMV IgG-negative donor recipient pairs (R\(^-\)/D\(^-\))

CMV-negative recipients of grafts from CMV-negative donors (R\(^-\)/D\(^-\)) very rarely develop major CMV-related complications and a CMV IgG-negative donor should be chosen in these circumstances when possible. There is debate about the relative importance of CMV serostatus and HLA compatibility in donor selection. Specifically, while HLA matching at HLA-A,B,C and DR remains the most important factor in donor selection, the value of choosing a CMV IgG-negative donor over mismatches at other loci, such as HLA DQ or DP, or in protocols with aggressive T cell depletion remains unresolved (Boeckh & Ljungman, 2009). It is also recognized that donor selection can be a complex process and optimum CMV matching may not always be possible (Kollman et al, 2001; Spellman et al, 2012). Provision of CMV-safe blood products is standard practice when both host and donor are negative, either derived from CMV IgG-negative recipients or achieved by leucodepletion. Both techniques are effective, although rare incidences of transfusion-transmitted infection have occurred using both approaches (Bowden et al, 1995; Ljungman et al, 2002b; Nichols et al, 2003). In the UK, universal leucodepletion has largely replaced blood products from CMV-negative donors as of May 2012. It is important to be aware of the need to check the baseline CMV IgG status of potential transplant candidates at the time of initial diagnosis, to avoid confusion caused by passive transmission of antibodies with transfusion of CMV IgG-positive blood products to negative recipients. Any transplant recipient who converts from CMV IgG-negative to CMV IgG-positive status pre-transplant will require careful assessment to separate passive antibody from true seroconversion, as this has major implications for donor selection. In this setting, a falling titre with time is suggestive of passively acquired antibody. Pre-allograft, it is recommended that samples for IgM antibody and CMV polymerase chain reaction (PCR) are sent as soon as a change in CMV status is suspected. If both are negative then this suggests the presence of passively acquired antibody. Blood products must be leucodepleted in the blood bank facility (Ratko et al, 2001) and evidence of quality control for leucoreduction should be available to all transplant centres for JACIE [Joint Accreditation Committee – International Society for Cellular Therapy (Europe) & European Group for Blood and Marrow Transplantation (EBMT)] accreditation purposes. R\(^+\)/D\(^-\) transplants still require to be monitored for CMV infection. Any cases of CMV infection occurring in this group of patients should be reported to the Serious Hazards of Transfusion (SHOT) scheme.

CMV IgG-positive donor or recipient

CMV seropositivity in either host or donor pre-transplant continues to be associated with a poorer overall survival post-allogeneic transplantation as a result of increased non-relapse mortality (NRM) (Broers et al, 2000; Kroger et al, 2001; Albano et al, 2006; Tomonari et al, 2008). The degree of this risk is determined primarily by the CMV IgG
status of the recipient. If a CMV IgG-negative recipient receives cells from a CMV IgG-positive donor (R+/D−) then CMV infection occurs in 20–30% of cases. CMV disease is unusual but NRM is increased, probably through the indirect effects of CMV on immune status post-transplant (Nichols et al, 2002; Ljungman et al, 2011; Pergam et al, 2012). There is a greater risk of CMV reactivation and progression to CMV disease in CMV seropositive recipients, where 80% are likely to reactivate CMV, irrespective of CMV status of donor (Ljungman et al, 2011). However, in most studies, major complications were further increased when the donor was CMV IgG-negative (Ozdemir et al, 2007; Zhou et al, 2009; Ugarte-Torres et al, 2011).

**Recommendations**

- All Potential HSCT recipients should be tested for the presence of CMV IgG antibody at diagnosis (Grade 1C).
- Once optimum HLA matching has been performed, a CMV IgG-negative donor should be chosen for a CMV IgG-negative recipient and a CMV IgG-positive donor should be chosen for CMV IgG-positive recipient when possible (Grade 1A).
- Donors or recipients who are initially found to be CMV IgG-negative should be retested pre-transplant to exclude primary CMV infection (Grade 1C).
- Apparent CMV seroconversion in potential allograft recipients who have received unscreened blood products should be actively investigated to exclude passively acquired antibody (Grade 1C).
- Any CMV IgG-negative HSCT recipient transplanted from a CMV IgG-negative donor who develops CMV infection post-transplant must be reported to SHOT (Grade 1C).

**Prevention of CMV disease occurrence**

Prophylactic and pre-emptive strategies have both been used to reduce the incidence of CMV disease. Universal monitoring of CMV levels in the blood is essential irrespective of whether prophylaxis is administered.

**Primary prophylaxis for CMV**

**Intravenous immunoglobulin**

There is no evidence that intravenous immunoglobulin (IVIG), CMV-specific hyperimmune immunoglobulin or anti-CMV monoclonal antibodies are useful alone or in combination with antiviral agents in primary prophylaxis against CMV infection (Bowden et al, 1991; Ruutu et al, 1997; Boeckh et al, 2001). A recent Cochrane review in solid organ transplant did not recommend prophylactic immunoglobulin (Hodson et al, 2007).

**Adoptive immunotherapy**

There have been several small studies published using CD4 or CD8 T cells (Walter et al, 1995; Einsele et al, 2002; Peggs et al, 2003, 2009; Hanley et al, 2011; Sili et al, 2012), or CMV peptide-loaded dendritic cell vaccination (Grigoleit et al, 2007) for treatment or prophylaxis of CMV infection, but too little evidence currently exists to make any recommendation at present although ongoing prospective studies are addressing this issue.

**Antiviral agents**

Aciclovir prophylaxis has been extensively studied post-HSCT. A large randomized trial of 310 patients initially appeared to suggest a reduced incidence and delayed onset of CMV infection as well as a significant improvement in survival. More mature follow up has shown no significant difference in CMV reactivation between groups, although reactivation did occur later in the prophylactic group. A modest survival benefit was still seen in the most aggressively treated patients, though it is difficult to attribute this to anti-CMV activity alone (Prentice et al, 1994, 1997). Improved survival in allograft patients who received aciclovir prophyaxis post-engraftment was also shown in a meta-analysis in which the vast majority of donor/recipient pairs were CMV IgG-positive (Yahav et al, 2009). However, the impact of aciclovir on CMV reactivation/disease rates in the studies included was again minimal and survival advantage was potentially mediated through anti-herpes simplex effects. Subsequently, valaciclovir, 2 g four times a day, was compared with oral aciclovir at 800 mg four times a day, in 727 patients following high dose intravenous (iv) aciclovir in the immediate post-transplant period (Ljungman et al, 2002c). In this study, valaciclovir significantly reduced CMV infection and disease rates (P < 0.0001). There was a 50% reduction in the use of pre-emptive therapy although there was no difference in overall survival (Ljungman et al, 2002c). Similar results were shown in a smaller case controlled study using valaciclovir at a dose of 1 g three times a day (Vusirikala et al, 2001). The studies described above predominantly used myeloablative conditioning and T-cell-replete BM as the stem cell source. Studies in PBSCT recipients, though smaller, suggested that the beneficial effects of high dose aciclovir prophylaxis appeared to be maintained (Verma et al, 2003; Hazar et al, 2004). However, aciclovir was shown to be significantly less effective in T-cell-depleted BM transplant recipients, where 83% of T-cell-depleted recipients still had a CMV reactivation compared to 41% of unmanipulated stem cell sources (P < 0.0001) (Nakamura et al, 2002). As most of these studies predated widespread use of pre-emptive therapy based on quantitative PCR, their significance is questionable in terms of current management of CMV, though a compelling argument can be made for its use for suppression of herpes simplex virus infection.
Ganciclovir prophylaxis significantly reduced the incidence of CMV infection and disease during the period of prophylaxis (Goodrich et al, 1993; Boeckh et al, 1996). However, neutropenia occurred in up to 30% of cases treated (Salzberger et al, 1997) and infective complications were increased (Boeckh et al, 1996). Ganciclovir was less effective in T-cell-depleted transplants and heavily immunosuppressed recipients (Maltezou et al, 1999). In a prospective randomized trial of ganciclovir versus aciclovir, cumulative rates of CMV disease were equivalent, although more patients in the aciclovir group required pre-emptive therapy ($P = 0.2$) (Burns et al, 2002). Post-prophylaxis, late onset CMV disease remained a problem (Boeckh et al, 2003) and prolonged exposure of CMV to ganciclovir, especially in the setting of T-cell depletion may encourage resistance, as occurs in solid organ transplantation (Eid et al, 2008). Valganciclovir prophylaxis has been reported to reduce risk of CMV disease in cord blood transplants (Montesinos et al, 2009).

More intensive prophylactic regimens involving pre-transplant ganciclovir combined with high dose aciclovir prophylaxis or a combination of ganciclovir and foscarnet have been employed in high-risk paediatric and cord blood transplants where both have been reported to be successful in reducing CMV infection and disease (Shereck et al, 2007; Milano et al, 2011).

Maribavir, when given from engraftment, initially showed favourable results in phase II studies; but, at the dose chosen, failed to show any effect on CMV disease or initiation of CMV pre-emptive therapy compared to placebo in a larger phase III study. There was a small impact on CMV DNA loads in plasma (Winston et al, 2008; Marty et al, 2011). Letermovir (AIC246), a maturation inhibitor of CMV, has been studied in phase 2 trials as anti-CMV prophylaxis in 133 HSCT patients with potentially encouraging results (Goldner et al, 2011). Neither of these drugs can be recommended for prophylaxis at present.

In summary, post-HSCT, in contrast to solid organ transplantation, ganciclovir-induced myelosuppression limits its use for prophylaxis. Routine use of aciclovir or valaciclovir is relatively non-toxic but will result in some patients being overtreated and the effect in T-cell-depleted transplants is small. However, the potential benefits of prophylaxis using these drugs in selected patients include reducing the need for hospital admission, for iv pre-emptive therapy, reducing indirect effects of CMV reactivation on immune status post-transplant and delaying CMV reactivation until the patient has recovered from the toxicity associated with the transplant and is no longer on immunosuppression.

**Secondary prophylaxis for CMV**

In patients who have had previous CMV disease prior to transplant or with recurrent episodes of CMV infection, especially in the context of T-cell depletion or GvHD, secondary prophylaxis should be considered, in conjunction with prolonged CMV viral screening. If prophylaxis is given, then oral valaciclovir 2 g three times a day or valganciclovir 900 mg daily is an option (Boeckh & Ljungman, 2009).

**Recommendations**

- **Primary prophylaxis with ganciclovir is not generally recommended as toxicity outweighs efficacy in HSCT patients (Grade 1B).**
- **Primary prophylaxis with aciclovir or valaciclovir can be deployed but only in conjunction with appropriate monitoring of CMV in the blood (Grade 1B).**
- **Valaciclovir or valganciclovir are valid treatment options for secondary prophylaxis with appropriate monitoring of CMV in the blood (Grade 1C).**
- **Intravenous immunoglobulins are not recommended for prophylaxis of CMV infection (Grade 1A).**

**Pre-emptive therapy**

The current mainstay for managing CMV infection after HSCT is the rapid introduction of therapy, based on evidence of CMV replication in blood (Goodrich et al, 1991). Success of pre-emptive therapy is dependent on the availability of a rapid, sensitive assay to allow early treatment at low levels of viral infection.

**Diagnostic tests for early detection of CMV infection**

Historically, the CMV antigenaemia assay provided a rapid way to detect CMV infection and is still used as a cost-effective screening tool in many centres worldwide. However, it suffers from low sensitivity, is not accurately quantitative and, in HSCT patients, is limited by the leucopenia evident in the early stages post-transplant. Here, CMV antigenaemia testing may be negative despite active viral replication (Gondo et al, 1994; Koehler et al, 1995). The availability of real time quantitative PCR (RQ-PCR) approaches has revolutionized the area of CMV DNA monitoring. RQ-PCR techniques provide rapid, high throughput platforms that are significantly less affected by white cell counts (Einsele et al, 1995; Emery et al, 2000; Fishman et al, 2007; Gimeno et al, 2008). The choice of sample type for routine monitoring lies between plasma and whole blood and remains controversial. The choice is often based on practical issues surrounding local sample handling and preparation. There are few published reports supporting the superiority of one sample type over the other. In the past, plasma was regarded as an ‘easier’ sample to extract, but this is now less of an issue as there are a number of automated extraction platforms that can handle whole blood. Several studies have shown that more CMV DNA can be extracted from whole blood samples than from plasma (Garrigue et al, 2006; Koidl et al, 2008; Bravo et al, 2011a). Responses to therapy may also be more predictable in
whole blood compared to plasma given the higher CMV DNA loads in the former (Atkinson & Emery, 2011). Other body fluids, where white cells may be scanty, such as urine, bronchoalveolar lavage, endotracheal aspirates, amniotic fluid and cerebrospinal fluid can also be rapidly analysed using RQ-PCR. However, to date, RQ-PCR has not been validated for diagnosis of CMV end-organ disease and no recommendations can be made for the use of this technology in this setting.

Methodological issues

A diverse range of in-house and commercially available RT-PCR assays are used in many diagnostic laboratories throughout the UK. The primers, probes and assay conditions used in these tests vary. Laboratories running in-house assays will regularly make up their own new reagents as part of the assay master mix, as well as a set of log dilutions of a separately amplified quantification standard, also known as a calibrator. This is used in order to produce a standard curve from which the number of copies of CMV DNA per ml of sample can be reported. A number of controls must be used when running a diagnostic PCR assay including:

- A positive control that is detected at the lower limit of the assay.
- At least one negative control to monitor for assay contamination.
- An internal amplification control that is an unrelated target to monitor for any substances inhibitory to the assay.
- A run control to monitor inter- and intra-variant variation.

Run controls are available commercially although some laboratories will produce their own controls. Standardization of nucleic acid extraction involves running an extraction control in each assay as well as an inhibition control for each sample. Recently, a World Health Organization standard for genome amplification of CMV from clinical samples was produced by the National Institute for Biological Standards and Control (Freyer et al, 2010; Bravo et al, 2011a). There are still significant differences in CMV DNA load values reported by different laboratories. These differences have previously been attributed to the variety of in-house methods used. However, it is likely that differences will still be seen using commercial assays as well. A recent comparison showed substantial differences in the nucleic acid extraction efficiency of a number of automated systems and differences in the commercial RQ PCR assays included in the study. This was most marked at the lower CMV DNA loads (Bravo et al, 2011a). It is likely that the international standard will be increasingly used to calibrate assays on a regular basis. This will allow CMV DNA levels to be compared between different centres and aid multicentre trials (Hirsch et al, 2013). Involvement with national external quality assurance schemes is strongly advised.

Timing of CMV screening samples

There is no clinical trial evidence demonstrating how often CMV DNA load in blood samples should be determined (Freyer et al, 2010). However, weekly assessment following allogeneic transplantation is advisable for the first 3–6 months (Hebart et al, 1997). The duration of monitoring will depend on the level of immunosuppression, the degree of immune reconstitution, the presence of GvHD and response to antiviral therapy. In these clinical settings, prolonged monitoring for 6–12 months may be required.

Recommendations

- Real-time quantitative PCR is the preferred option for monitoring CMV DNA levels in HSCT patients (Grade 1B).
- All diagnostic laboratories should deploy the CMV international standard to allow CMV DNA loads to be compared between centres (Grade 1C).
- Monitoring of CMV DNA load should be undertaken at least weekly for the first 3 months post-HSCT (Grade 2C).
- CMV viral load monitoring should continue to 6–12 months if the patient has chronic GvHD or prolonged T-cell immunodeficiency (Grade 1B).

Treatment thresholds

There is no consensus concerning the CMV load at which pre-emptive treatment should be initiated (Boeckh & Ljungman, 2009; Ljungman et al, 2011). Some centres will start antiviral therapy once CMV DNA has been detected at any level in two consecutive samples, while others have set much higher thresholds on the basis of local assays and clinical outcome data. Unfortunately, these thresholds will remain difficult to compare between centres until PCR testing is fully standardized. A randomized trial comparing antigenaemia with RQ-PCR has indicated that a CMV load >10 000 copies/ml may be an acceptable threshold for initiation of therapy. (Gerna et al, 2008; Boeckh & Ljungman, 2009; Mullier et al, 2009). This allows some patients to generate effective immune responses and avoid toxic drug therapy while still treating susceptible patients pre-emptively, before the onset of CMV disease. Demonstration of active CMV-specific T-cell responses may help select patients who do not require treatment (Cwynarski et al, 2001; Hebart et al, 2002; Ozdemir et al, 2002). In addition to the peak viral load there is evidence that the initial viral load and the rate of viral load increase are important predictors of development of CMV disease (Emery et al, 2000). Risk-adapted strategies are likely to be needed and early treatment is advised in R+/D- patients, especially in those with severe lymphopenia, high initial viral loads (e.g. >3–4 log10 copies/ml), rapid viral load doubling times (e.g. >1 log10 per day)·
Drug Side effect Treatment/support
Ganciclovir Myelosuppression Granulocyte colony-stimulating factor
Valganciclovir Haemolysis Toxicity-specific dose reductions Regular twice daily SHT3 inhibitors
Electrolyte abnormalities Aggressive electrolyte replacement Fluid support Regular twice daily SHT3 inhibitors Toxicity-specific dose reductions
Renal impairment Nausea/vomiting Genitourinary Urinary symptoms
Cidofovir Renal impairment Nausea/vomiting Ocular

Ganciclovir. Ganciclovir requires phosphorylation by the UL 97 viral kinase. It is virustatic. It inhibits viral replication by competing with deoxyguanosine triphosphate as a substrate for viral DNA polymerase (UL 54) (Sia & Patel, 2000). Resistance is mediated by mutations in genes encoding either of these enzymes (Chou, 2008). Ganciclovir has been extensively used to treat CMV infection and disease (Goodrich et al, 1991; Manteiga et al, 1998). It is still regarded as first choice pre-emptive therapy in most HSCT centres (Pollack et al, 2011). The standard dose is 5 mg/kg intravenously twice daily for 14 d with maintenance of 5 mg/kg daily for a further 7–14 d. Side effects and supportive therapies are listed in Table I.

Valganciclovir. Valganciclovir is the valine ester of ganciclovir, it is hydrolysed to ganciclovir after oral absorption. A small randomized trial in HSCT patients showed that oral valganciclovir 900 mg twice daily led to higher effective bioavailability of active drug compared to iv ganciclovir 5 mg/kg bd and showed equivalent efficacy (Lim et al, 2009). Good absorption was also shown in the context of intestinal GvHD grade I–II (Einsele et al, 2006). Large prospective randomized trials comparing valganciclovir and ganciclovir are lacking in HSCT patients. However, there are now several single centre randomized studies published, including over 150 patients, comparing the use of oral valganciclovir 900 mg bd with 5 mg/kg bd iv ganciclovir. All these studies showed no difference in any measure of efficacy although the power of each individual study to exclude non-inferiority was limited (Einsele et al, 2006; van der Heiden et al, 2006; Lim et al, 2009; Chawla et al, 2011; Ruiz-Camps et al, 2011). Further evidence of valganciclovir effectiveness was derived from several single arm case series. These studies reported response rates comparable to historic controls using ganciclovir (Ayala et al, 2006; Rzepecki et al, 2008; Liu et al, 2010). CMV resistance rates appeared low following pre-emptive therapy with valganciclovir for CMV in HSCT (Allice et al, 2009). In solid organ transplants, where valganciclovir was used extensively for prophylaxis it was shown to be non-inferior to ganciclovir in preventing both CMV infection and disease, with low rates of CMV resistance (Andrews et al, 2011). After pre-emptive therapy in solid organ transplants the kinetics of decrease in viral load were the same as for intravenous ganciclovir (Mattes et al, 2005).

Foscarnet. Foscarnet does not require phosphorylation and is virustatic through inhibition of viral DNA polymerase (Sia & Patel, 2000). Two randomized studies have compared the outcomes of foscarnet versus ganciclovir pre-emptive therapy of CMV infection post-allograft (Moretti et al, 1998; Reusser et al, 2002). Foscarnet 90 mg/kg was compared with ganciclovir 5 mg/kg, both administered 12 hourly iv, using CMV antigenaemia as a guide to commencement of therapy (Moretti et al, 1998). Of 40 patients were randomly allocated to either treatment. There were no statistically significant differences in rates of resolution of antigenaemia, treatment failure or progression to CMV disease (5–10% in both groups). Both drugs required dose reductions of at least 20% in approximately half of all patients. A larger EBMT study compared 60 mg/kg foscarnet (n = 110) with ganciclovir 5 mg/kg (n = 103) both 12 hourly using either RQ-PCR or antigenaemia as a trigger to commencement therapy (Reusser et al, 2002). No differences in CMV disease occurrence, treatment-related mortality (TRM) or event-free survival were seen. Renal insufficiency was more common with foscarnet and myelosuppression with ganciclovir. Further evidence of foscarnet efficacy comes from case series. In one, 313 patients were treated for CMV disease (n = 65), or were given pre-emptive therapy of CMV reactivation in related- donor transplants (n = 248) (Asakura et al, 2010). Of 194 patients had failed previous ganciclovir due to lack of efficacy (n = 99) or myelosuppression in (n = 95). Fifty two percent of patients with

Recommendations

- Each transplant centre should have a risk-adapted policy detailing threshold values for treatment of CMV infection, taking into account patient factors and local PCR methodology (Grade 2C).

Antiviral agents used in pre-emptive therapy

Ganciclovir. Each transplant centre should have a risk-adapted policy detailing threshold values for treatment of CMV infection, taking into account patient factors and local PCR methodology (Grade 2C).
Guideline

CMV disease and 90% of pre-emptively treated cases responded. More limited data were reported supporting foscarnet use in cord blood transplantation (Narimatsu et al, 2007; Takami et al, 2007).

Cidofovir. Cidofovir is a nucleotide analogue that does not require phosphorylation by viral UL97 kinase for activation. The largest case series using cidofovir has been published by the infectious disease working party of EBMT (Ljungman et al, 2001). 82 patients were treated as primary (n = 24) or secondary (n = 38) pre-emptive therapy for CMV infection or for CMV disease (n = 20). Most patients were treated with 5 mg/kg per week (range 1–5) for a median duration of 3 weeks. Response rates were 50% for CMV disease, 66% for primary and 62% for secondary CMV infection. Other smaller series demonstrated that cidofovir was effective but response rates varied from 50 to 90% with most series reporting significant failure rates (Chakrabarti et al, 2001; Platzbecker et al, 2001; Cesaro et al, 2005). Side effects are shown in Table I. A more tolerable alternative dosing regimen of 1 mg/kg three times a week has been used to treat patients with adenovirus infection (Lindemans et al, 2010) but there is no information using this strategy for the treatment of CMV. Modified hexadec oxypropyl-cidofovir (HDP-CDV/CMX001), or other liposomal preparations of cidofovir have been developed but their use has only been studied in small numbers of patients (Hostetler, 2010; Bravo et al, 2011b; Gokulgandhi et al, 2012) and no recommendations are possible.

Combination anti-viral drug therapy

The theoretical advantages of in vivo combination therapy are reduced toxicity, and increased efficacy, both demonstrated in vitro (Manischewitz et al, 1990; Manion et al, 1996): the primary disadvantage is generation of resistance, particularly in the T-cell-deficient, immunocompromised host, post-allograft. The combination of foscarnet and ganciclovir (both at half dose) versus full dose ganciclovir was evaluated in a small randomized study (n = 48) in transplant patients, including HSCT recipients (Mattes et al, 2004). There was less myelosuppression but overall increased toxicity, primarily renal, in the combination arm. Importantly – though underpowered to show a clinical effect – 71% of the ganciclovir group reached the primary clinical endpoint of viral clearance at 14 d compared to 50% in the combination group. At present, not enough evidence exists to support treatment using these two drugs at sub-conventional doses. Bacigalupo et al (1996a) have also reported combining foscarnet and ganciclovir at full dose, adjusted for organ function, aiming for 180 mg/kg/d foscarnet and 10 mg/kg/d ganciclovir and delivering 50–60% of these doses. Drugs were delivered together at full dose for 2 weeks followed by maintenance, where drugs were given at full dose but on alternate days. This approach was effective and the authors suggested a possible reduction in TRM compared with historical controls (Bacigalupo et al, 1996b). However, not enough evidence exists to make a recommendation regarding combination therapy in this context.

Recommendations

- Ganciclovir is recommended as first line pre-emptive therapy for CMV in HSCT patients (Grade 1A).
- Oral valganciclovir is a useful alternative when gastrointestinal absorption is normal or minimally impaired (Grade 1B).
- Foscarnet is recommended as an alternative first line agent if neutropenia is present or for ganciclovir treatment failures (Grade 1A).
- Pre-emptive therapy with cidofovir can be considered as third line in patients unresponsive or intolerant of a ganciclovir preparation or foscarnet (Grade 2B).

Switching pre-emptive therapy

The doubling time of CMV DNA load in untreated patients is 1–2 d on average but may be even more rapid (Emery et al, 1999; Buyck et al, 2010) At the start of pre-emptive therapy, before drugs can have a major impact, increases in CMV DNA load occur in approximately one-third of patients due to underlying immunosuppression and rapidity of replication (Buyck et al, 2010; Park et al, 2011). This should be appreciated and no changes in therapy instituted in the first 14 d, in the absence of overt CMV disease or dramatic rises in viral PCR log values. It is difficult to be dogmatic about triggers for changing pre-emptive antiviral drugs, as responses to treatment can be very slow in profoundly lymphopenic patients. An increase in viral load by one log after 2 weeks of therapy is one option. Switching therapy is seldom recommended earlier, and can often be delayed further, even in high-risk patients, unless disease has progressed clinically, or viral copy number is increasing rapidly.

Recommendations

- In patients where CMV DNA loads in blood increase by 1 log₁₀ over 2 weeks of pre-emptive therapy with a first line drug, an alternative agent and drug resistance profiling should be considered (Grade 2C).

Antiviral drug resistance

Drug resistance is relatively uncommon in the stem cell transplant setting. Resistance to ganciclovir is estimated at 2–8% of treated patients and generally occurs after 2–3 months of prolonged therapy (Marfori et al, 2007) but can occur after initial therapy (van der Beek et al, 2012; Kim et al, 2012).
Close liaison between transplant physicians and clinical virologists is essential. Resistance is less common in drug-naive patients than in patients experiencing repeated episodes of late onset CMV infection. Resistance should be suspected in patients on antiviral drugs where the CMV DNA load has been static or has increased for more than 2 weeks, although ‘clinical’ resistance is still more likely in lymphopenic drug-naive patients. If drug resistance is suspected, samples should be sent for CMV UL97 and UL54 sequencing.

**Recommendations**

- Drug resistance should be considered if the CMV DNA load in blood fails to respond after 14 d of therapy, especially in non-lymphopenic or multiply pre-treated patients (Grade 2C).
- Sequence analysis of the UL97 and UL54 genes is the preferred option for monitoring resistance to currently available drugs (Grade 1B).

**Management of CMV disease**

A multidisciplinary approach to the management of CMV disease is important. Specific investigations and treatment depends on which organs are affected and whether disease has developed *de novo* or from asymptomatic CMV infection while on first-line therapy. *De novo* CMV disease can be treated with ganciclovir 5 mg/kg, or foscarnet 90 mg/kg twice daily for 2 weeks, followed by maintenance, although more prolonged treatment may be necessary. Careful ophthalmological review is mandatory as intraocular therapy may be required (Song et al., 2008). Higher baseline CMV DNA loads, faster kinetics of replication and a slower viral decay rate after starting therapy are associated with increased rates of treatment failure (Mates et al., 2004) and mandate closer monitoring of these patients with lower thresholds for diagnostic interventions. If CMV disease occurs during first line pre-emptive therapy or if *de novo* CMV pneumonia is progressive at any time, then alternative strategies should be considered. Failure to respond is more likely to be related to the host’s inability to control the virus as a result of impaired T cell immunity than to drug resistance, unless multiple courses of therapy have been given previously (van der Beek et al., 2012). However, in critically unwell patients, where available, drug resistance testing should be performed early to optimize pharmacological management.

Alternative treatment approaches in this setting include increasing ganciclovir doses to 7.5 mg/kg twice a day (Beczk & Ljungman, 2009) or switching ganciclovir to foscar-


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