**The best practices for diagnosis and monitoring of CMV infection in immunocompromised patients**

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**ABSTRACT**

Human cytomegalovirus (CMV), an ubiquitous member of the Herpesvirus family, is an important pathogen for humans. CMV is rarely pathogenic in healthy adult but is associated with several diseases in immunocompromised individuals (such as HIV-infected subjects and transplant recipients).

CMV infection in transplant recipients may cause different clinical syndromes and the severity of the infection parallels the degree of the immunosuppression. Not only does CMV directly cause morbidity and occasional mortality, it also influences many short- and long-term indirect effects that collectively contribute to reduce allograft and patient survival.

To prevent CMV infection and disease, two major therapeutic approaches are currently employed, antiviral prophylaxis and preemptive therapy. Both strategies demonstrated efficacy and safety.

As infections are either asymptomatic or accompanied by symptoms that are not specific of CMV (such as fever and leukopenia), laboratory techniques are the sole means of diagnosing CMV infection. Diagnosis of CMV infection can be made directly by demonstration of the virus or virus components in pathological materials or indirectly through serology.

Systemic CMV infections are diagnosed by performing quantitative antigenaemia or DNAemia assay on blood samples. Local infections are diagnosed by CMV identification from tissue biopsies and/or secretions.

CMV serology is confined to the pre-transplant diagnosis of CMV infection in immunocompromised patients.

In future, simultaneous virological and immunological follow-up will be the best approach to efficient monitoring of CMV infections in immunocompromised patients.

**INTRODUCTION**

Human cytomegalovirus (CMV) is one of the eight viruses belonging to the Herpesviridae family to infect humans. CMV belongs to the Betaherpesvirinae subfamily of viruses characterized by a restricted host spectrum, in vitro replication in fibroblasts of the natural host species in vivo, a slow replication cycle, the induction of intranuclear and intracytoplasmic inclusions and the ability to induce latency mainly in the myeloid cell lineage (Mocarski 2007).

CMV infection is endemic and ubiquitous and not subject to seasonal fluctuations. During their lives from 40 to 80% of individuals in the industrialised countries and almost all those in the developing world will be infected by CMV. The seroprevalence of CMV infection increases with age in every group that has been studied (Ho 1990).

Human beings are deemed to be the only reservoir for human CMV. The virus is transmitted by direct contact and only indirectly in rare cases. Because of viral fragility towards environmental factors, close contact is required for horizontal propagation of infection. Sources of infection include: oropharyngeal secretions, urine, cervical and vaginal secretions, sperm, breast milk, tears, faeces and blood (Waner 1977, Handsfield 1985, Barbara 1987). Propagation is fostered by prolonged elimination of the virus and the fact that most infections produced few or no symptoms.

Transmission of the virus may be vertical (from mother to child via the placenta) or horizontal (by infectious CMV in excreta and iatrogenically by blood transfusion or transplanted organs and cells).

CMV is rarely pathogenic in healthy adult but is associated with several diseases in immunocompromised individuals (such as HIV-infected subjects and transplant recipients).

The clinical importance of this virus has grown in
parallel with the increasing number of immunocompromised individuals.
As infections are either asymptomatic or accompanied by symptoms that are not specific of CMV (such as fever and leukopenia), laboratory techniques are the sole means of diagnosing acute CMV infection. Diagnosis of CMV infection can be made directly by demonstration of the virus or virus components in pathological materials or indirectly through serology.

IMMUNOCOMPROMISED PATIENTS
The most severe infections are seen in recipients of allogeneic bone marrow or stem cell transplant and in AIDS patients with low CD4+ T cells counts. CMV symptomatic infection are also often observed in solid organ transplant recipients.
CMV retinitis and colitis are common features among AIDS patients with a CD4+ T cell count below 100 cells/mm³. Prior to the use of combination regimens of highly active antiretroviral agents (HAART), it was estimated that around 40% of adults and 9% of children with AIDS would develop CMV disease (Shepp 1996). The incidence of CMV retinitis and disseminated disease has dramatically decreased with the advent of HAART, as a result of the increase in CMV-specific immunity coupled with the decrease in CMV reactivation (Varani 2000, Springer 2004)
Consequently, after the introduction of HAART in the developed world, CMV disease afflicts only a limited number of HIV-infected patients, when CD4+ cell counts fail to rise or when reconstitution of specific CMV-immune response does not occur.
Although the incidence of CMV retinitis is reduced, patients with AIDS and CMV retinitis remain at increased risk for mortality, retinitis progression, complications of the retinitis, and visual loss over a 5-year period (Jabs 2010).

CMV INFECTION IN TRANSPLANT RECIPIENTS
Rejection and infection are the two main obstacles to successful in solid organ and hematopoietic cell transplantation in the short and long-term, and both processes are closely associated and interdependent. Adjustments to immunosuppressive protocols to deal with rejection favour the onset of infection. In turn, reduced immunosuppression fosters infection. More than two thirds of transplant recipients experience at least one episode of infection in the first year after transplant (Fishman 2007, Snydman 2001, Kotton 2008).
CMV infection is one of the commonest opportunistic infections of varying aetiology arising in recipients of solid organs (Razonable 2003) hematopoietic stem cells (Ljungman 2008).
While CMV may cause asymptomatic infection in transplant patients, it may progress to full-blown clinical disease especially in those with severely compromised immune function.
The direct clinical effect of CMV results from its replication, its subsequent dissemination in the blood invasion of target organs. These direct viral effects, which may occur during primary infection, reactivation or reinfection, produce a clinical picture characterized by flu-like febrile illness and bone marrow suppression (an entity termed “CMV syndrome”) (Razonable 2010). Almost all transplant recipients develop a CMV infection, but only 30-50% of them will have clinical repercussions in the absence of prophylaxis or preemptive treatment. CMV disease may give rise to a broad spectrum of clinical manifestations (Table 1), and may also carry a high risk of acute rejection and hence a life-threatening illness for transplant patients. In more severe cases, CMV disease is characterized by end-organ disease manifestations, the most common organ involved is the gastrointestinal tract (in the form of gastritis, enteritis and colitis) (Razonable 2010).
Additionally, CMV is implicated in short-term and long-term indirect effects that threaten allograft and patient survival. CMV is associated with chronic rejection episodes including obliterating bronchiolitis in lung transplant recipients (Haﬁkin 2009) and vanishing bile duct syndrome following liver transplantation (Razonable 2008). Accumulating evidence suggests that systemic CMV infections in heart transplant recipients arising in the early weeks after transplantation may trigger the onset of graft atherosclerosis and chronic rejection (Potena 2009).
Early primary CMV infections are also deemed a risk factor for the progression of HCV infection towards disease and the onset of post-transplant EBV-associated lymphoproliferative disorders (Ljungman 2008).
Two types of infection occur in transplantation:
1. Primary infection in subjects who have never come into contact with the virus during their lives and hence have no specific CMV immunity
2. Reactivation of primary infection in subjects who have been previously infected and have reactivated the virus.

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<th>Symptom</th>
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+/-: little prevalent; +: prevalent; ++: very prevalent
SOT, solid organ transplant; HSCT, haematopoietic stem cell transplant;
AIDS, acquired immunodeficiency syndrome.

Tabella 1: Cytomegalovirus diseases in the immunocompromised patient.
(adapted from Razonable Ref. 15)
when they undergo transplantation of an organ or tissue from a CMV seropositive donor (negative recipient/positive donor: \(D+/R^-\)); in the vast majority of CMV \(D+/R^-\) transplant recipients, primary CMV infection and disease will develop if the patient does not receive CMV prevention strategy.

Hematopoietic stem cell transplant recipients develop primary CMV infection in about 30% and have an increased mortality in bacterial and fungal infections (Ljungman 2008).

2. Non-primary infection (reactivation/reinfec-
tion) is encountered in subjects who are CMV seropositive prior to transplantation (\(D-/R^+/D+/R^+\)).

The rate of CMV disease in CMV \(D+/R^+\) is higher compared with CMV \(D-/R^+\) transplant recipients (Manuel 2009).

Both primary and non-primary CMV infection often manifests following increases in the immunosuppres-
sive protocol to control rejection, especially when anti-lymphocyte drugs are used (ATG, ALG, OKT3) (Fishman 2007).

**INFECTION SURVEILLANCE**

Complex diagnostic problems arise in transplant recipients even when active viral infection is ascertained as the illness is often asymptomatic in these patients. For this reason it is essential to make use of diagnostic tests offering information on predictive factors for dis-
ease onset, control factors for disease progression, and hence factors that can monitor the efficacy of antiviral therapy or promptly disclose treatment failure.

It is now well-established that the critical point of CMV infection leading to the development of symp-
toms is blood diffusion made possible by viral replication in the endothelium. The blood phase precedes or coincides with a generalized diffusion of infection and can be controlled by quantitative tests as the amount of CMV (or its components) in the blood is directly proportional to the extent of infection/disease (Perciv-
alle 1933, Grefte 1993).

In addition, special attention must be paid to post-
operative monitoring of CMV infection in bowel and lung transplant recipients as these patients undergo intense prolonged immunosuppressive treatment due to the frequent severe episodes of acute rejection (Hafkin 2009, Abu-Elmagd 2009). As a result, CMV infections in these patients, whatever the serologi-
cal status of recipient/donor, are unusually severe and life-threatening and are deemed the main cause of acute enteritis and pneumonia. For reasons that remain unsettled, the transplanted organ is the main target of CMV infection and disease. In the case of bowel transplantation the impaired permeability of the intestinal mucosa favours blood diffusion of CMV possibly involving other organs. Bowel and lung transplant recipients must be strictly monitored for long periods checking not only the blood phase but also the trans-
planted organ.

Prevention, prompt accurate diagnosis, appropriate management of infection and other complications are the main objectives of clinical and laboratory trans-
plant medicine aimed at reducing mortality rates not correlated to recurrence of the underlying disease.

In recent years, these aims have led to the development and improvement of clinical strategies and specific sensitive laboratory tests applicable in particularly sort time frames.

**DIAGNOSIS AND MONITORING OF CMV INFECTION**

**Virological Findings**

Virological tests are the best means of establishing diagnosis of CMV infection and monitoring of CMV infection in solid organ transplant (SOT) and haemato-
opoietic stem cell transplant (HSCT) recipients, during the post-transplant period (Gerna 2006). In cases of disseminated CMV infection, blood is the specimen of choice. Local organ samples (secretions or tissue biopsies) are the best specimens to use in cases of organ syndromes either separate from or associated with systemic infection, as revealed by the absence or presence, respectively, of CMV in blood.

**Viraemia:** rapid viral isolation by the shell vial pro-
dure will detect and quantify infectious viral particles transported by polymorphonuclear leukocytes (granu-
locytes, neutrophils) (Figure 1). A positive test result indicates viral replication is underway but given its limited sensitivity, positive viraemia is generally held to reflect either an untreated infection or the onset of a resistant viral strain during treatment with antiviral drugs.

Viral culture of blood has a limited role for the diagno-
sis of disease (Kotton 2010).

**Antigenaemia:** this method will identify and quantify peripheral blood leukocytes transporting the CMV pp65 (ppUL83) structural phosphoprotein that is pro-
duced in abundance and endowed with marked trop-
ism for the cell nucleus (Figure 2). Peripheral blood samples must be used promptly (within 6 to 8 hours of sampling – as neutrophils are not stable for longer periods) as delays lead to a major reduction in the number of positive cells. The test is based on four basic steps: 1) dextrane separation of peripheral blood PMNL and slide preparation (cell centrifugation); 2) PMNL fixation and permeabiliza-
tion; 3) detection by immunostaining with anti pp65 monoclonal antibodies and fluorescin or enzyme label-
ing; 4) reading and quantification of the results. The assay may not be possible when the absolute neutrophil
count is less than 1,000 neutrophils/μl.
Since antigenaemia was first described many studies have attempted to correlate the number of pp65-positive cells with the onset of clinical signs to establish a threshold at which treatment should be started irrespective of symptoms (Grossi 1995). Despite individual exceptions, years of experience and hundreds of published papers indicate that 50-100 positive cells out of 200,000 PMNL is a major predictive parameter of increased risk for development of disease in solid organ transplant recipients (Grossi 1995, Gerna 1998, Egan 1995), whereas a lower number of pp65-positive cells is indicative of risk for development of CMV disease onset in haemopoietic stem cell recipients (Locatelli 1994, Gerna 2003, Ljungman 2008). In addition, infections occur soon after stem cell transplantation and are exacerbated by the fact that immunosuppression is administered to patients who already have an immunosuppressive illness and reach surgery in aplasia.
Once treatment has been instituted, antigenaemia tends to subside over a period of days except when ganciclovir is given, as it often is during primary CMV infection to solid organ transplant recipients and infections arising in haemopoietic stem cell recipients. Clinical observations and subsequent in vitro studies have demonstrated that even when the patient responds to antiviral treatment antigenaemia can continue to increase for one to two weeks to then subside gradually to become negative (Gerna 2003). This has never been observed when treatment is monitored by vireaemia or DNAemia testing. The main reason for this atypical response is probably due to the lack of a pre-existing cell-mediated response in CMV negative transplant recipients and a prolonged delay in recovering this immune response in stem cell recipients. Ganciclovir acts by inhibiting viral DNA and thereby blocking elongation of the viral DNA. For this reason the drug is deemed an inhibitor of CMV DNA synthesis. Under certain conditions of impaired cell-mediated response, some infected PMNL may escape the drug’s action while the abundant pp65 protein in the cytoplasm is readily transferred to other cells (Gerna 2003). In conclusion, antigenaemia quantification does not directly correlate with actual viral replication, providing misleading information in certain cases. The antigenemia test has advantages in some settings, because it does not require expensive equipment and the assay is relatively easy to perform. There are problems with a lack of assay standardization, including subjective result interpretation (Kotton 2010).

**DNAemia:** this test will identify and quantify viral DNA in peripheral blood. Viral DNA assays were first developed in the early 1990s for both the detection and quantification of CMV DNA in blood specimens by PCR (Polymerase Chain Reaction). Quantitative PCR assays were then developed in the format of real-time PCR assays, which are now the most commonly used diagnostic method. Most laboratories are moving to these new technologies, as they have better precision, broader linear range, faster turnaround time, less risk of carry over contamination than conventional PCR tests and, not least a standardization program is possible (Drew 2007).
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 CMV DNA quantification (Wolff 2009).

Fourteen Italian viral diagnostics laboratories belonging to different transplantation centers participated in the external Quality Control Programme for Molecular Diagnostics CMV-DNA by using two in-house and five commercial methods for CMV-DNA quantification. The different method shared 100% specificity, and sensitivity reached 100% when samples containing >1,000 copies/ml were considered. The variability range was within ±0.5 log₁₀ for samples containing a CMV-DNA levels ≥ 5,000 copies/ml (Lilleri 2009).

Again in 2009, Pang and colleagues reported the results of interlaboratory comparison of viral load assays to assess variability in qualitative and quantitative CMV testing. This study included 33 laboratories from Canada, USA and Europe who performed testing using commercial reagents (n=17) or laboratory-developed assays (n=18) (Pang 2009). This study indicates that only 57.6% of results fell within acceptable standards for CMV viral load detection. Variability in commercial assays was lower than that for laboratory-developed assays. The greatest variation was in the sensitivity or lower limits of detection for each assay, which must be sufficient to allow early detection and treatment of infection while avoiding false positive tests or the detection of clinically irrelevant levels of infection (Fishman 2009).
Finally, Caliendo and colleagues observed that a comparable CMV calibrator is required to improve the agreement of viral load values between laboratories (Caliendo 2009).
It is evident that creation of an international reference standard for CMV viral load assay calibration would be an important step in quality improvement of this laboratory toll.

The World Health Organisation (WHO) Expert Committee on Biological Standardisation has endorsed a proposal to develop the 1st WHO International Committee on Biological Standardisation has endorsed a proposal to develop the 1st WHO International Standard for CMV for the calibration of nucleic acid amplification technology (NAT)-based assays.

The candidate is a whole virus preparation of the CMV Merlin strain, formulated in a universal buffer comprising Tris-HCl and human serum albumin, and freeze-dried for long-term stability. Thirty-two laboratories from 14 countries participated in collaborative study experiments to evaluate the performance of the candidate material in NAT assays. The WHO Expert Committee on Biological Standardisation has endorsed a proposal to develop the 1st WHO International Standard for CMV for the calibration of nucleic acid amplification technology (NAT)-based assays.
to evaluate the fitness for purpose and potency of the candidate standard using their routine NAT-based assays for HCMV. The results of the study indicate the suitability of the candidate HCMV Merlin standard as the proposed 1st WHO International Standard for HCMV. The final report will be presented at the 3rd SOGAT Clinical Diagnostics Meeting which will be held in London, January 2011. Viral DNA can be quantified in different blood compartments (leukocytes, plasma/serum, or whole blood), but several studies have shown that whole blood is the specimen of choice for CMV DNAemia quantification, since it allows determination of both cell-free and cell-associated virus (Razonable 2002). Plasma and whole blood specimens both provide prognostic and diagnostic information on CMV disease, but whole blood is more sensitive for the PCR diagnosis of CMV infection. The first studies suggested that whole blood would represent the optimal sample for CMV DNA quantification (Razonable 2002). Only one specimen type should be used when serially monitoring patients. Quantification of the number of CMV DNA copies will determine the dynamics of viral replication in infected patients and hence serve to make decisions regarding pre-emptive therapy, monitor antiviral therapy and establish drug efficacy and identify any strains resistant to antiviral drugs. The testing requires expensive equipment and reagents, specialized expertise (Kotton 2010).

**Diagnoses for end-organ disease:** In more severe cases, CMV disease is characterized by end-organ disease manifestations such as pneumonitis, gastritis, enteritis, colitis, encephalitis, hepatitis, retinitis and others (collectively called tissue-invasive CMV disease). The clinical suspicion for tissue-invasive CMV disease is confirmed by the demonstration of CMV in tissue specimens by PCR, histopathology, immunohistochemistry or in situ DNA hybridization (Kotton 2010, Fica 2007). In the vast majority of tissue-invasive CMV diseases, the virus is also demonstrated in the blood. In a minority of cases, however, the illness is ‘compartmentalized’ and there is no detectable virus in the blood (Ruel 2007); in these cases, a tissue biopsy is the optimal means to confirm the diagnosis of tissue-invasive disease (Fica 2007).

CMV organ localization are diagnosed by examining organ biopsies or, alternatively, local secretions (e.g. bronchoalveolar lavage fluid (BAL) for pulmonary CMV infection, cerebrospinal fluid (CSF) for central nervous system disease). For BAL, CSF and biopsy specimens, laboratories should be moving toward quantitative real-time PCR because it provides quantitative results, improved sensitivity without loss of specificity. At present, there are no randomized clinical data regarding the interpretation of quantitative PCR viral load testing in BAL specimens, although several studies suggest it may be helpful in predicting pneumonitis (Kotton 2010).

Gastrointestinal disease was the most frequent end-organ disease in small bowel/multivisceral transplantation and intestinal biopsies taken during ileoscopy from the intestinal graft is the best biological samples (Ganzenmueller 2009). Petrisil and colleagues in 2009 demonstrated that CMV replication must be monitored both in blood and biopsy samples in intestinal transplant recipients. The elective assay for monitoring viral load in these patients is quantitative determination in blood and biopsy specimens by real-time PCR because it directly correlates with CMV replication and clinical symptoms, allowing more effective antiviral therapy or judicious weaning of immunosuppression (Petrisil 2010). The diagnosis of retinitis is based on ophthalmologic examination; viral load in blood or plasma or other laboratory tests are rarely useful as predictors of CMV eye disease although they may be positive before and at the time of diagnosis of CMV retinitis (Kotton 2010).

**SEROLOGICAL FINDINGS** Serological procedures are confined to the pre-transplant diagnosis of CMV infection in immunocompromised patients. They serve to search for CMV-specific IgG in serum to identify CMV seronegative patients at risk of contracting primary infection and CMV seropositive patients at risk of reactivations and/or reinfections.

CMV serology should be performed pretransplant on both the organ/tissue and the recipient. A test that measures anti-CMV IgG should be used, because IgG serologic tests have better specificity than IgM tests or tests combining IgG and IgM (Kotton 2010).

**PREVENTION OF CMV INFECTION AND DISEASE** Two major therapeutic approaches are currently employed to manage CMV infections and diseases: universal prophylaxis and pre-emptive therapy using the currently available anti-CMV compounds.

Both strategies demonstrated efficacy and safety for the prevention of CMV diseases after transplantation. The pre-emptive therapy reduced the incidence of CMV disease by 70%, while the prophylaxis by 60-80% (Hodson 2008, Kall 2005, Small 2006). The drugs most commonly used for the prevention of CMV disease are ganciclovir (GCV) and valganciclovir (VGCV), while foscarnet (PFA) and cidofovir (CDV) are not routinely used because of common occurrence of potentially severe (Kotton 2010).

In prophylaxis, the antiviral drug is administered before active CMV infection is detected to prevent its occurrence and involves all patients or a sub-set of at risk patients. Antiviral are usually begun in the immediate post transplant period and continued for a finite period of time, often in the range of 3-6 months. The therapeutic value of an antiviral prophylaxis in transplant recipients is controversial and may be useful in some cases, but it is a high-cost strategy that exposes all patients to drug toxicity (marrow suppression and renal toxicity), the selection of GCV or VGCV resistance, and the risk to develop of late onset or relapsing CMV disease after discontinuation (Snydman 2005).

The most basic tenant of and the theory behind pre-emptive treatment is that a small rate of CMV replication is a good thing. The suppression of viral replication with prophylaxis may reduce the likelihood of indirect effects of CMV disease, such a graft rejection and opportunistic infection. Its potential interfering role with a delay in HCMV-specific T-cell reconstitution is still debated (Snydman 2005).

Antiviral prophylaxis is generally recommended for patients at high risk of CMV disease such as lung, intestinal and pancreas recipients, and all CMV D+/R-solid organ transplant recipients. Prophylaxis should be adopted only in transplantation centres with no
facilities for virological monitoring (Kotton 2010). **In pre-emptive therapy**, a shorter course of antiviral treatment is targeted toward a subset of patients with early viral replication in an attempt to prevent the progression of asymptomatic infection to symptomatic full-blown CMV disease. The success of pre-emptive therapy hinges on timely detection of CMV infection. The logistics of conducting surveillance tests are widely perceived to be a major barrier to the implementation of pre-emptive therapy.

Antiviral treatment is initiated one viral replication reaches a certain threshold in the blood and/or organ using sensitive methods, such as Real Time PCR and tests for viral antigen. The advantages for pre-emptive strategy include the targeting of antiviral therapy to those most at risk for future disease, reducing the number of patients exposed to antiviral toxicity, lowering the risk of drug resistance, and maximizing the cost:benefit ratio (Singh 2005). In fact, 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 (Singh 2005).

It is generally recommended to treat transplant recipients until all the laboratory evidence of CMV replication has resolved. It is common practice to provide treatment until the virus is no longer detected for at least 2 consecutive weeks in the blood (Kotton 2010. Humar 2009). Therefore, in pre-emptive therapy, laboratory monitoring is performed at regular interval to detected early and asymptomatic viral replication, but a universal cut-off for antigenemia or DNA blood-organ viral load for initiating therapy has not been established. Individual laboratories should establish their own reference cut-off and verify the results with clinical outcome.

**Virological monitoring** should be performed CMV DNAemia quantification on whole blood weekly during the first three months after transplantation. When active CMV infection is diagnosed (i.e. positive DNAemia) more frequent monitoring (2 tests/week) should be performed (Gerna 2006). Beyond three months after transplantation (or six months), in order to avoid onset of late CMV disease, monitoring should be performed: 1) monthly (or at least in concomitance with routine medical visits); 2) in case of an increase in the immunosuppressive regimen due to rejection; and 3) on the basis of any clinical indication suggesting the presence of CMV infection/disease. In case of an active CMV infection, weekly or biweekly monitoring should be reinstated.

In lung transplant recipients, CMV-DNA monitoring in BAL in concomitance with routine bronchoscopy procedures for rejection surveillance is suggested. In small bowell/multivisceral transplant recipients, CMV-DNA monitoring in intestinal biopsy samples taken during routine ileoscopy to monitor the rejection status is suggested.

**Immunological monitoring.** Adaptive immune response of B and T lymphocytes are critical in controlling CMV replication. B cells are important in the umoral reponse to CMV, producing neutralizing antibodies that primarily target glycoprotein B (gB) and H (gH) (Crought 2009). The T-cell-mediated immune response is the major factor responsible for the control of CMV infection in both immunocompetent and immunocompromised patients. Reconstitution (or development) of CMV-specific cellular immunity post transplantation is a critical determinant of the control of CMV infection, both the CD4+ and the CD8+ arms of T-cell immune response must develop in order to achieve a sustained control of CMV infection while the absence of T-cell immunity is consistently associated with the risk of incurring repeat episodes linked to a recurrent infection.

Several, yet not standardised, techniques are utilized to monitor CMV-specific CD4+ and CD8+ T-cell immune responses. The majority of assays rely on the detection of IFN-γ after stimulation with CMV specific antigens (Crought 2009, Sester 2005, Gandhi 2004). Direct T-cell stimulation by peptides relevant to the immunodominant epitopes of CMV viral proteins such as pp65 (ppUL83) and pp72 (ppUL123) (Maeker 2001), or stimulation by whole crude viral antigen, may provide satisfactory results. An alternative, stronger stimulatory effect may be achieved by incubating viral antigens or peptides with autologous dendritic cells. Responsiveness to these challenges can be measured by interferon production using cytokine flow cytometry and human leukocyte antigen tetramer staining or by the enzyme linked immunospot (ELISPOT) technique (Figure 3). The number of responsive cells can be calculated as a percentage of the total CD4+ or CD8+ population. To facilitate quantitation of CMV specific lymphocytes test kits are now commercially available which allow intracellular cytokine staining (Drew 2007).

Study recently completed in both SOT and HSCT recipients show that the reconstitution of both CD4+ and CD8+ specific T-cell immunity is capable of con-
trolling CMV infection in absence of antiviral treatment, whereas a delayed immune response allows development of repeated episodes of recurrent infection, which can only be controlled by antiviral treatment, until both arms of the T-cell response are reconstituted (Gerna 2006, Singhal 2000, Lilleri 2006, Gerna 2006a, Chiereghin 2010).

The role of virus-specific immune response monitoring in transplanted patients management should be verified in future prospective studies (research need). In future, the best approach to monitoring transplanted patients for CMV infections is to perform a careful virological and immunological follow-up in all patients. Assays of CMV specific lymphocytes after transplant can identify patients at higher risk for CMV disease who require intensive monitoring or prophylaxis.

LATE-ONSET CMV DISEASE

Late-onset (also termed delayed-onset) CMV disease is as significant complication in transplant recipients receiving antiviral prophylaxis, particularly among CMV D+/R- solid organ transplant patients. The determinants of late-onset CMV disease in patients receiving prophylaxis have not been fully elucidated, but are likely related to ongoing significant immunosuppression, accompanied by a lack of development of significant CMV specific cell-mediated immunity (Kotton 2010).

The estimated incidence of late-onset CMV disease varied in different studies, from a low as 8% to as high as 47% of CMV D+/R- solid organ transplant recipients who received 3 months of prophylaxis. The onset of late-onset CMV generally occurs between 130 and 160 days after transplantation, or the first 3 months after cessation of prophylaxis (Razonable 2010). Late CMV disease is associated with a mortality rate of 46% and a recurrence in 38% of survivors in hematopoietic stem cell transplant recipients receiving ganciclovir prophylaxis either at engraftment or pre-emptively in response to blood viral load (Boeck 2003). The severe immunosuppression of the transplant recipients (CD4+ counts lower than 100 cells per mm³) remains the most important risk factor for infection and subsequent late CMV diseases. The common manifestations of CMV disease in these patients are interstitial pneumonia and gastrointestinal disease. Recently, some authors reported that CMV CNS disease is a late-onset disease (median time of onset, 210 days) after allogeneic hematopoietic stem cell transplantation. Encephalitis alone is the predominant clinical manifestation.

The development of CMV CNS disease is associated with risk factors (T-cell depletion, anti-thymocyte globulin, umbilical cord blood transplantation) that cause severe and protracted T-cell immunodeficiency, a history of recurrent CMV viremia treated with multiple courses of preemptive ganciclovir or foscarinet therapy, and ganciclovir-resistant CMV infection (Reddy 2010, Candoni 2010).

ANTIVIRAL DRUG RESISTANCE

The major mechanism underlying phenotypic resistance to ganciclovir are mutations in UL97 phosphotransferase and/or UL54 DNA polymerase genes. The incidence of ganciclovir-resistant CMV disease remains very low, although it seems to be higher in lung compared with other organ transplants (Razonable 2010). Antiviral drug resistance is suspected when increasing or high-level CMV viraemia or progressive clinical disease is observed during prolonged antiviral therapy. The increases in viral loads, especially in the first weeks of treatment, are not reliable indicators of drug resistance. Accurate diagnosis requires diagnostic laboratory testing (Kotton 2010).

REFERENCE