



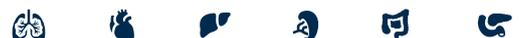
The Voice of Transplantation in the UK

The Prevention and Management of CMV Disease after Solid Organ Transplantation

—
Third Edition

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British Transplantation Society Guidelines



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Disclaimer

These Guidelines are guides to best practice which inevitably change with the passage of time. All practitioners need to undertake clinical care on an individual basis and keep themselves up to date with changes in practice of clinical medicine. The British Transplantation Society Guidelines ("the Guidelines") were compiled by a working party of the Society. The Guidelines represent the collective opinions of a number of experts in the field and do not have the force of law. The Guidelines contain information and guidance for use by practitioners as a best practice tool; it follows that the Guidelines should be interpreted as such rather than the letter of their contents. The opinions presented in the Guidelines are subject to change and should not be considered to be a treatment recommendation for any individual patient.

The British Transplantation Society cannot attest to the accuracy, completeness or currency of the opinions contained herein and does not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on the Guidelines or as a result of any inaccurate or misleading opinion contained in the Guidelines.

Grading of Recommendations

In this guideline, the GRADE system has been used to rate the strength of evidence and the strength of recommendations (1). This approach is consistent with that adopted by KDIGO in its recent guidance relating to renal transplantation, and also with guidelines from the European Best Practice Committee, and from the UK Renal Association.

For each recommendation the quality of evidence has been graded as one of:

A (high)

B (moderate)

C (low)

D (very low)

For each recommendation, the strength of recommendation has been indicated as one of:

Level 1 (we recommend)

Level 2 (we suggest)

Not Graded (where there is not enough evidence to allow formal grading)

Summary of Recommendations

For Prevention

All organ donors and recipients should be screened for CMV status prior to, or at the time of transplantation (1 A)

Where both donor and recipient are seronegative for CMV, leukodepleted blood and blood products should be used to minimise the risk of primary infection (1 A)

In this situation, no prophylaxis or monitoring is required (1 B)

CMV seronegative recipients who receive a solid organ transplant from a donor who is seropositive should be offered prophylaxis against primary infection. The same should apply where either the donor or recipient is seropositive if the patient is treated with T-cell depleting antibodies.

For renal transplant recipients, the recommended management strategy is one of:

Oral valganciclovir for at least 100 days (1 A), *or*

Oral valganciclovir for 200 days (2 B)

Oral valaciclovir for 90 days (2 B)

Intravenous ganciclovir for 28 days (2 A)

Serial measurements of viral load and treatment with oral valganciclovir or intravenous ganciclovir when levels predictive of disease are reached (2 A)

For liver transplant recipients, the recommended management strategy is one of:

Oral valganciclovir for 100 days (1 A)

Intravenous ganciclovir for 100 days (2 B)

Serial measurements of viral load and treatment with oral valganciclovir or intravenous ganciclovir when levels predictive of disease are reached (2 A)

For kidney/pancreas transplant recipients, the recommended management strategy is one of:

Oral valganciclovir for 100 - 200 days (1 C)

Intravenous ganciclovir for 28 days (2 C)

Oral valaciclovir for ninety days (2 C)

Serial measurements of viral load and treatment with intravenous ganciclovir when levels predictive of disease are reached (2 B)

For lung transplant recipients, the recommended management strategy is one of:

Oral valganciclovir for 100 - 360 days (1 B)

For heart transplant recipients, the recommended management strategy is one of:

Oral valganciclovir for 100 days (1 A)

Intravenous ganciclovir for 28 days (2 B)

Intravenous ganciclovir followed by oral valganciclovir for 60 days (Level 2 B)

Serial measurements of viral load and treatment with intravenous ganciclovir when levels predictive of disease are reached (2 C)

When the donor and recipient are both seropositive and the patient is not treated with T cell depleting antibody therapy:

For renal transplant recipients, no prophylaxis is recommended (1 A)

For liver transplant recipients, no prophylaxis is recommended (1 A)

For renal / pancreas transplant recipients, no prophylaxis is recommended (1 C)

For lung transplant recipients, the recommended prophylactic strategy is one of:

Oral valganciclovir for 100 days (1 C)

Oral valaciclovir for 90 days (2 C)

For heart transplant recipients, no prophylaxis is recommended (1 C)

In these cases serial measurements of viral load and treatment with intravenous ganciclovir when levels predictive of disease are reached can be deployed (Level 2 B)

For Treatment

Patients with CMV disease should receive intravenous ganciclovir or oral valganciclovir until resolution of symptoms and for a minimum of 14 days (1 B)

Foscarnet and cidofovir are second line therapeutic options unless ganciclovir resistance has been demonstrated (Not graded, B)

Consideration should be given to a reduction in immunosuppression (Not graded, D)

After treatment doses have been administered, an additional 1-3 months of appropriate prophylaxis should be considered to minimise the risk of recurrent infection (Not graded, D)

The duration and efficacy of treatment should be determined using PCR monitoring of viral load (1 B)

Drug Availability

Oral ganciclovir is no longer available in the United Kingdom. However, some of the literature relating to oral ganciclovir is summarised because of the possibility in the future for the manufacture of a generic product. The option to use the agent has been removed from the overall recommendations.

Guideline Development

The management of post transplant CMV disease has been previously reviewed. Web-based guidelines from the International Herpes Management Forum were published in 2004. These included advice on measuring viral load as well as prophylaxis and pre-emptive management in both solid organ transplant recipients and recipients of haemopoietic stem cell transplants (2). The recommendations of a consensus workshop were published in 2005 (3). The Cochrane collaboration has published three systematic reviews on antiviral medication for preventing CMV disease in solid organ transplant recipients, the most recent in 2008 (4). In 2009, both the American Society of Transplantation/American Society of Transplant Surgeons and KDIGO published guidelines on several aspects of solid organ transplantation including cytomegalovirus infection (5, 6). In 2010, International consensus guidelines on the management of CMV in solid organ transplantation were published under the auspices of the Transplantation Society (7).

Guidelines for the management of post transplant CMV were first produced by the British Transplantation Society in 2001. The first draft was written by Dr CG Newstead (Consultant Nephrologist, St James's University Hospital, Leeds). Major revisions were made by Prof PD Griffiths (Professor of Virology, Royal Free Hospital and University College Medical School, London) and less extensive revisions by Dr JG O'Grady (Consultant Hepatologist, Institute of Liver Studies, Kings College Hospital, London) and Dr KJ Parameshwar (Consultant Cardiologist Transplantation, Papworth Hospital, Cambridgeshire). A revised second edition was published by the same authors in 2004. In 2010, Dr CG Newstead was asked to start a revision which would lead to a third edition. His draft was extensively revised

by Professor VC Emery (Professor of Virology, UCL Medical School) and Dr PA Andrews (Consultant Nephrologist, St Helier Hospital, Surrey; Chair of the BTS Standards Committee). Contributions to this draft were also made by Dr JH Brown (Consultant Nephrologist, Belfast City Hospital, Northern Ireland) and Dr KJ Parameshwar (Consultant Cardiologist Transplantation, Papworth Hospital, Cambridgeshire). The last date of literature review was October 2010. The revised document was circulated to members of the BTS Council for comment and placed on the BTS web site for one month in February 2011 to allow comments from interested parties. A revised version was placed on the website in March 2011. The final version of the guideline was published in August 2011 following receipt of comments received in a second round of open consultation.

These guidelines represent consensus opinion from experts in the field of transplantation medicine in the United Kingdom. They represent a snapshot of the evidence available at the time of writing. It is recognised that recommendations are made even when the evidence is weak. It is felt that this is helpful to clinicians in daily practice and is similar to the approach adopted by KDIGO (6). Although it is believed that the information presented is a fair summary of current evidence and best practice, neither the authors nor the British Transplantation Society can be held responsible for any errors or omissions. The guidelines are not designed to be proscriptive, nor to define a standard of care. Doses of prescribed drugs should always be checked by the responsible clinician according to the relevant information provided by the manufacturers of the drugs.

It is anticipated that these guidelines will next be revised in 2015.

Biology of CMV in Man

Cytomegalovirus is one of the herpes group of viruses which are widely distributed among mammals. The various strains of CMV are species specific and produce a cytopathic effect resulting in greatly enlarged (cytomegalic) cells containing cytoplasmic and intranuclear inclusions. The primary infection results in the most severe disease especially when the host is T-cell immunocompromised. After primary CMV infection the viral genome enters monocytes and other bone marrow progenitor cells and enters a latent state, although it is likely that frequent reactivations occur which are not associated with disease in healthy individuals. Re-infection with a different human strain can also occur and in simian CMV models this is facilitated by the presence of the immune evasion genes encoded by all CMV strains analysed to date (8).

In this guideline we use the term latent to define a state of virus infection in which the full replication cycle of the virus is not occurring; whereas active infection is defined as a state there is evidence of the virus undergoing a complete replication cycle and producing new infectious virions. Active replication can be further characterised into asymptomatic infection (no obvious signs of pathologic symptoms), viral syndrome (fever $>38^{\circ}\text{C}$ for 2 days of unexplained origin and one of leucopenia, myalgia or arthralgia) and CMV disease (histopathological evidence of CMV, CMV retinitis diagnosed by an ophthalmologist, or CMV in the CSF indicative of CNS disease).

Re-infection usually results in less serious disease than the primary infection. Reactivation may be provoked by immunosuppression due either to another disease such as carcinoma or

AIDS, or treatment with immunosuppressive or chemotherapeutic agents, and usually results in a more clinically benign course compared to primary infection.

The prevalence of antibody indicating previous infection increases with age in all human populations that have been studied. The prevalence of past exposure to CMV, as indicated by a positive IgG, varies markedly throughout the world and is close to 100% in adults in many developing countries such as the Philippines and Uganda (9). In the developed world the percentage of the population who are seropositive increases roughly linearly with age and is approximately 40% at age 20 and 80% at age 60 (10, 11).

Transmission occurs from direct person-to-person contact. As the virus is labile intimate exposure to saliva, urine, breast milk or genital secretions has to occur and the risk of transmission to health care workers is very low. Although congenital CMV infection only rarely results in disease, it may cause hepatosplenomegaly, jaundice, microcephalae, prematurity, choroidoretinitis, petechiae, mental retardation and hearing loss and is an important health care burden. Indeed, there is a growing appreciation that neonates born with asymptomatic congenital infection remain at increased risk of hearing loss in the early years of life (12). Perinatal infection is more common but clinically benign. In the immunocompetent child or adult primary infection is usually sub-clinical. Malaise, fever and myalgia are the most frequent symptoms, with biochemical hepatitis and atypical lymphocytes found on investigation.

The Diagnosis of CMV Infection and Disease

A challenge in the transplant recipient is differentiating patients with active ongoing CMV infection who are at risk of CMV disease from those who have a latent infection. Primary infection with CMV typically occurs approximately four to six weeks post-transplantation in a seronegative individual who receives a seropositive organ. Symptoms due to primary disease may occur as early as 20 days and are rare more than 50 days post-transplantation provided the patient has not received antiviral drugs (13). Many symptoms such as fever, night sweats, fatigue and myalgia are non-specific. Retinitis can be pathognomonic, but is rarely seen in the transplant population. Respiratory distress noticed at first on exercise is a sinister symptom and measurement of oxygen saturation and blood gas analysis, the former at both rest and exercise, can give an early clue to pulmonary involvement. Gastrointestinal disease presenting with diarrhoea, abdominal pain and nausea is common. One group has suggested that epigastric pain that decreases in the supine position is a symptom uniquely seen in CMV gastritis (14). Symptomatic adrenal insufficiency is unusual, possibly because many transplant recipients receive supra-physiological doses of corticosteroids.

The detection of the classic large cells in culture takes several weeks and so is of little practical clinical use. Routine blood tests may detect bone marrow suppression, especially of the white cells, as well as biochemical hepatitis evidenced by fluctuations in ALT and AST. After a primary infection an individual would be expected to mount IgM and later an IgG immunoglobulin response against CMV. In immunocompetent individuals the presence of IgM and/or low avidity IgG antibodies is a hallmark of primary infection. In the immunosuppressed, however, the antibody rise may be delayed or absent and these tests are

at best only of use for retrospective diagnosis. The presence of IgG is used to determine prior exposure of both donors and recipients to CMV infection, most commonly at the time of transplant wait listing, and of donors at the time of organ retrieval but it is of little value after transplantation.

Early antigen fluorescent foci fixation (DEAFF) tests rely on the detection of CMV generated antigen in cells in urine or alveolar macrophages obtained from direct lavage. As with all fluorescent based techniques, subjective interpretation is problematic and false positive results are seen (15). As positive results from bronchoalveolar lavage in lung transplant recipients may only indicate viral secretion and not disease (16) and as urine culture has very poor specificity (17) it is now rare for this test to be used to guide clinical management in those specimens. The demonstration of CMV p65 antigen in circulating polymorphonuclear leukocytes in the buffy coat may discriminate between infection and disease, but subjective interpretation and poor reproducibility present problems, particularly when delay occurs in the processing of specimens (18). The test is, however, semi-quantitative and has been used to both monitor response to therapy as well as a guide to starting treatment (19, 20). The fact that the test does not require expensive equipment and is relatively easy to perform is an advantage.

Tests based on the polymerase chain reaction (PCR) carried out on plasma, whole blood or leukocytes are much more sensitive and have become the gold standard in many laboratories in the UK and beyond. In the early days of using this technique, technical problems with contamination and inhibition, as well as the lack of standardisation between laboratories, had to be overcome (21). However, increased experience and the availability of commercial

assays have now allowed PCR diagnosis and monitoring to be offered on a routine basis (22, 23). Real time PCR offers highly reproducible data on viral load to be obtained rapidly and has become increasingly used (24).

CMV load, used as a surrogate marker of CMV replication, has been shown in many studies to be a dominant risk factor for CMV disease. Approaches that maintain viral replication at very low levels in the early post-transplant period (prophylactic therapy) or which rapidly reduce viral load when a certain level has been reached (pre-emptive therapy) have become the principal methods of controlling CMV disease. Uncertainty whether it is best to test plasma or whole blood and the lack of an international reference standard has made the comparison of cut-offs for the initiation of antiviral therapy difficult to compare between laboratories and problematic when patients migrate between different care centres. At the time of writing, the NIBSC UK has completed a study which should result in the distribution of a WHO genomic standard for CMV in 2011 which will alleviate many of these problems.

On occasion it is necessary to prove CMV organ specific dysfunction by obtaining a biopsy. This can be especially useful if co-infection with another organism is suspected or if another cause of allograft dysfunction, such as rejection, is within the differential diagnosis. Liver, native and allograft, bone marrow, lung, renal allograft and gastrointestinal biopsies can be diagnostic. The usual clinical approach is to choose to biopsy an organ that is demonstrating clear dysfunction, balancing the risk of the diagnostic procedure against the likelihood of an unequivocal result. CMV is a systemic infection and histological diagnosis may be achieved from unlikely sites (25). Immunohistochemistry staining of cells obtained from

bronchoalveolar lavage may be helpful, although quantitative PCR testing of such specimens is increasingly used (26).

Histopathology may be specific for CMV disease by identifying CMV inclusion bodies, or suggestive as in the detection of 'microabscesses' in the parenchyma in CMV hepatitis. In either event, histopathology is insensitive compared to PCR (27). The detection of CMV inclusions in an organ biopsied because of characteristic symptoms and signs meets the internationally agreed case definition of 'CMV disease' (28). More recently, enhanced sensitivity has been achieved with immunohistochemistry, illustrating that CMV may be more prevalent in organs of transplant patients than previously recognised, although this technique is not routinely deployed in most histology laboratories. The data from enhanced immunohistochemistry are consistent with findings using in situ hybridisation showing that CMV genomes are frequently detected in transplanted organs many weeks prior to the occurrence of CMV in the blood (29). The key objective of modern management is to avoid patients reaching a clinical endpoint of CMV syndrome or tissue invasive disease.

CMV Frequency and Manifestations in Solid Organ Transplant Recipients

Frequency of CMV in Transplant Recipients

The frequency of CMV disease varies markedly depending on the definition of CMV disease that is used and the intensity of immunosuppression. Approximately 8% of renal, 29% of liver, 25% of heart and 39% of lung transplants can be expected to experience symptomatic CMV infection (30).

With the proviso regarding the definition of CMV disease already mentioned, there is only modest literature regarding the impact of CMV disease in transplant patients from the era before effective prophylaxis. Snyderman et al showed a 30% death rate in seronegative recipients of seropositive kidneys who received Anti-Lymphocyte Globulin (see 31). An economic analysis of the impact of CMV disease in liver transplant recipients demonstrated that the development of CMV disease was associated with a 49% increase in costs and that effective antiviral prophylaxis was associated with an overall reduction in costs in the CMV seronegative recipient CMV seropositive donor combination (32). An audit from Manchester UK showed that 30% of renal recipients were high risk and that half of these experienced CMV disease. An average of eight in-patient days per 'at risk' patient were spent managing CMV disease post-transplantation (33). The frequency of disease among the positive recipients of positive kidneys (D+/R+) and positive recipients of negative kidneys (D-/R+) was extremely low. In a publication describing liver transplant recipients who did not receive antiviral prophylaxis, 8 out of 9 donor positive/recipient negative (D+/R-) and 7 out of 17 donor negative/recipient positive (D-/R+) patients developed CMV disease (34).

Because of the multiple human strains of CMV, seropositive organ recipients are at risk of re-infection with a different strain of virus (35). In this situation, the clinical syndrome is usually less severe than in primary infection and the onset of disease is often delayed to approximately 6-8 weeks post-transplantation. Seropositive recipients of seronegative grafts (and seronegative blood products) can also develop CMV disease due to reactivation of latent virus. This is usually relatively mild compared to primary infection and also often delayed to approximately 6-8 weeks post-transplantation. Leukodepleted or filtered whole blood has a very low (essentially negligible) risk for transmission of CMV infection (36).

Cytomegalovirus and Early Allograft Dysfunction

CMV infection may decrease cell-mediated immunity, reducing the T-helper to suppressor cell ratio as well as the ability of T-cells to produce interferon- γ . This may allow coincident infection with other viral, bacterial, protozoal or fungal organisms. Despite the immunosuppressive effects of acute CMV disease, it has long been recognised that CMV infection can be coincident with acute allograft rejection (37). Prophylaxis with valganciclovir reduced biopsy-proven acute graft rejection by 50% in the D+/R- subgroup of renal transplant recipients (38). CMV increases the expression of major histocompatibility (MHC) class I and II molecules on both vascular endothelial and tubular epithelial cells which are targets for renal allograft rejection. The mechanism may be via the production of interferon- γ by T-cells (39, 40) as well as the increased expression of MHC molecules. Another mechanism of enhanced rejection may be the fact that CMV encodes molecules similar to MHC class I antigens and that there is some homology between the immediate early region protein of CMV and some class II antigens.

As well as these effects, which would be expected to enhance the alloantigen dependent rejection, CMV infection would be expected to enhance the alloantigen independent pathway of rejection by increasing co-stimulatory molecules on antigen presenting cells, vascular endothelial cells, tubular epithelial cells and T-lymphocytes (41, 42). Elevated anti-endothelial cell antibodies and IL-2 levels have been reported in a small group of renal and cardiac allograft recipients coincident with CMV infection. This may indicate an increased humoral response to endothelial antigens, which the authors postulated could be a risk factor for what was termed at that time both vascular and chronic rejection (43). Using sensitive in situ hybridisation techniques for CMV genomes, extensive infection of renal tubular cells, and to a lesser extent, endothelial cells has been described in early renal biopsies. In some cases infection preceded acute rejection, whereas in others it was either not associated with concurrent acute rejection or was coincident with the acute rejection. Both studies showed that long term graft function was significantly poorer in patients with intra-graft CMV. (44). These data illustrate the challenge of deciding the appropriate management of acute rejection in the context of CMV infection.

There has been considerable interest regarding the potential role of CMV infection in both native coronary and cardiac allograft atherosclerosis. In 60 histological specimens of restenoses after native coronary angioplasty, 38% were found to have accumulated a high amount of tumour suppresser protein p53 and this correlated with the presence of CMV in the lesions (41). In the rat aortic allograft model of chronic vascular rejection, early infection with rat CMV doubled the rate of smooth muscle proliferation and arteriosclerotic alterations in the intima, while late infection had almost no effect (42). In this model, immunosuppression had a protective (rather than detrimental) effect on vascular wall histology (45). In a whole organ model in the rat, CMV significantly enhanced the

development of renal chronic allograft rejection (46). In other experiments, again in the rat allograft model, treatment with ganciclovir blocked the early adventitial inflammation and reduced smooth muscle cell proliferation (47).

A *post-hoc* analysis of a subset of a randomised placebo controlled study reviewed 149 consecutive heart transplant patients who received either intravenous ganciclovir or placebo for the initial 28 days after transplantation (48). The patients underwent annual arteriography. Twenty-eight could not be evaluated, mostly because of early death. The rest had a mean follow up of 4.7 years. The actuarial incidence of transplant coronary artery disease was 43% vs 60% in the ganciclovir treated and control groups respectively. One of the independent risk factors for coronary artery disease was no ganciclovir treatment (relative risk 2.1, confidence interval 1.1-5.3, $p=0.04$). However, the report has considerable limitations, as the authors emphasise, in that it was not designed to address the specific question explored in the more recent paper.

Despite the intriguing *in vivo* animal and human data, there is *in vitro* work showing that the induction of cell surface adhesion molecules after CMV infection is not influenced by ganciclovir (49). Further data from the same group at Stanford demonstrated differences in cardiac vessel integrity between patients receiving standard prophylaxis (intravenous ganciclovir for 4 weeks) or enhanced prophylaxis (CMV hyperimmune globulin plus 4 weeks intravenous ganciclovir followed by 2 months valganciclovir 900mg once daily). Patients in the aggressive treatment arm also had decreased acute rejection (RR 0.55, $p=0.03$), reduced coronary artery lumen loss (-10% vs -21%, $p=0.05$) and reduced vessel shrinkage (-3% vs -11%, $p=0.03$). Although these data make an important contribution, it was unfortunate that

enhanced prophylaxis was only given to the high risk D+/R- group rather than all patients being randomized to the two treatment arms (50).

A study of 60 liver transplant recipients having at least four blood specimens for PCR analyses showed that both CMV and HHV6 were associated significantly with acute graft rejection (51). In another complex study, 242 consecutive renal transplants were prospectively followed, including 157 with and 85 without CMV infection (52). The latter group were randomly paired with 85 of the infected patients and given matched dates for fictitious CMV infections. The outcome was that the incidence of acute rejection after CMV infection was higher among those infected patients (45% vs 11%).

Cytomegalovirus and Late Allograft Dysfunction

A clinical study predating the availability of effective antiviral therapy found a strong association between CMV infection (rather than disease) and chronic rejection after liver transplantation (53). Two other studies strongly linked CMV hepatitis and failure to clear CMV from the liver with chronic rejection (54, 55). However, no difference in the incidence of chronic rejection was found during the first year after liver transplantation in a large multicentre study of oral ganciclovir prophylaxis (13). The significance of this observation has been questioned by some authorities because of the short period of follow-up.

In a series of 301 heart transplant recipients, 91 showed serological evidence of CMV infection and graft atherosclerosis was more frequent and occurred earlier in this group. Ninety percent of the non-CMV patients were free of angiographically severe obstruction compared with 72% of the CMV patients (56). In 128 heart transplant recipients who were transplanted between 1992 and 1993, all of whom received four weeks of intravenous

ganciclovir and then oral aciclovir, early severe rejection was associated with CMV viraemia (47 vs 16%) and tissue-invasive CMV (11 vs 0%). However there was no association between either symptomatic CMV or asymptomatic CMV and the subsequent development of allograft vasculopathy over the subsequent 6 to 7 years (57). The potential impact of acute CMV disease on chronic vascular rejection will need examining further by prospective studies.

Large registry data, as well as single centre studies, demonstrate reduced graft and patient survival in patients who experience CMV disease, and also those who are at highest risk of primary infection. In the UNOS database, which includes over 47,000 patients, the renal graft survival disadvantage in the cohort whose donor was CMV seropositive was 4% at three years (58). Some single centres have reported even more deleterious outcome (59), but others have shown no such effect (60).

Cytomegalovirus and Graft and Patient Survival

An attempt was made to separate the impact of acute rejection and CMV disease on long term graft survival in a single centre study of 1,339 renal transplant recipients. A multivariate analysis showed that CMV disease appeared to influence long term graft survival but only when coupled with the occurrence of acute rejection (61).

In a liver transplant population of 33 patients receiving 57 transplants, persistent CMV infection as defined by serial PCR measurements was significantly associated with graft loss through chronic rejection. However, there was no significant correlation between primary infection or symptomatic disease and chronic rejection, possibly as a consequence of the small sample size (62).

Interesting data with regard to the incidence of CMV infection on long-term outcome comes from a series of 1,545 cadaveric renal transplant recipients divided historically into two groups on the basis of availability of ganciclovir. In the early group, the survival of the D+/R- patients was significantly poorer (63). However, close inspection of the survival curves shows no change in late graft survival before and after the use of universal prophylaxis, arguing against a causative role for CMV infection in late graft loss after renal transplantation. A recent publication examined graft outcome in 10,190 adult and paediatric renal transplants performed in the UK between 2000-7. After adjustment for donor age, this showed no significant effect of donor or recipient CMV status on either allograft or patient survival at three years post transplant (64).

Registry data which is regularly updated that examines patient survival is available from the Collaborative Transplant Society website, <http://www.ctstransplant.org/>. There is approximately an 8% difference in survival at five years in the case of D+/R- renal transplant recipients transplanted between 1985 and 2008 between those who did and did not receive prophylaxis (data set K-71302-0810). For liver transplantation, there is an approximately 5% survival advantage for D+/R- recipients who received prophylaxis, seen at one year (data set L-71312-0810); a similar result is shown after pancreas transplantation (data set P-71302-0810). In lung transplantation, the data is even more striking with CMV prophylaxis resulting in 95% vs 44% survival at three months in those who did and did not receive CMV prophylaxis (65).

Two recent studies in renal transplant recipients have investigated the relationship between CMV infection of the graft and long term graft function (66, 67). Both studies show that

CMV can be identified in the graft at an early stage post transplantation; that patients with intragraft CMV are likely to proceed to an episode of CMV DNAemia; and that they also have worse long term graft function (assessed by creatinine levels) at 1 year post transplant.

Indirect Effects of Cytomegalovirus Infection

As well as acute illness due to CMV disease and effects on allograft function there has been increasing interest in what have been termed ‘indirect effects’ of CMV infection. These can be defined as effects (or often associations) that are not explained by tissue invasion by the virus. As well as the potential role in premature cardiovascular disease discussed above, an increase in serious infections co-incident with CMV disease has long been recognised (68). The co-tissue localization of CMV virus with colo-rectal (69) and prostatic cancer (70) and the association of CMV viraemia with diabetes (71) are examples of potential indirect effects. Asymptomatic CMV viraemia in renal transplant recipients was associated with a three times higher risk of death after a median follow up of about five years (72) and may be explained by immunosuppressive or inflammatory effects of the virus or alternatively that viraemia is a marker for other aspects of ‘ill health’. The failure in the past to detect CMV in affected tissues most likely reflects the sensitivity of the detection methods available. With the advent of in situ hybridisation for CMV DNA, PCR analysis of biopsies and the deployment of improved immunohistochemistry methodology it is clear that CMV is very often present in diseased organs. These data therefore challenge the concept that the indirect effects may not be due to virus presence per se. It could be that many of what were thought to be indirect effects may be due to a direct influence of the virus on tissue architecture or cytokine networks, and hence the immune response to the infection.

Prevention of CMV disease

CMV Matching

In theory, one method to minimise the risk of CMV infection would be to avoid transplanting a seropositive organ into a seronegative recipient. Historically, before the advent of oral antiviral prophylaxis, many units avoided transplanting CMV positive lungs into CMV negative recipients. However, given the shortage of donor organs and the life-sustaining nature of cadaveric heart, lung and liver transplantation, such an approach is difficult to practise in these settings.

In renal or liver transplantation, this approach would be possible in the rare situation where there was more than one equally good candidate for a CMV seronegative cadaver organ; where a seronegative recipient had several potential living donors of different CMV status; or where an unusual degree of immunosuppressive therapy was anticipated, such as ABO incompatible transplantation. In practice, however, modern antiviral therapy means that other factors usually predominate. There is also the theoretical concern that the widespread adoption of CMV matching would disadvantage younger recipients as they are more likely to be CMV negative, and could compromise HLA matching or other criteria that are currently used to determine organ allocation.

One area where CMV matching remains relevant is in the elective use of blood products. Where it is known that both donor and recipient are seronegative for CMV, leukodepleted blood and blood products are available and should be used to minimise the risk of primary infection.

Vaccination

Another theoretically attractive option for prevention would be vaccination against CMV. However, the heterogeneity of strains has limited the yield from vaccination as a prophylactic strategy. Early attempts using the Towne virus strain in renal transplant recipients were largely unsuccessful although there were some effects on the severity of CMV disease (73). More recently vaccines directed against envelope glycoproteins have generated renewed interest as these are well conserved between strains. A recombinant glycoprotein B vaccine produced in CHO cell by Sanofi-Pasteur has been shown to prevent infection in seronegative women (74) and the same vaccine has undergone a phase II safety and immunogenicity study in renal and liver transplant recipients at the Royal Free Hospital / UCL London. The biggest public health return would be achieved by vaccinating women prior to the reproductive age (75). A DNA vaccine has been shown to be safe and to generate an antibody response in healthy volunteers (76) and this is being studied in haemopoietic transplantation with encouraging results (presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy) (www.clinicaltrials.gov). In addition, an alphavirus non-replicating vaccine incorporating glycoprotein B and a pp65-IE1 fusion protein (representing major targets for CD8 T-cell responses) has been subjected to phase 1 studies with impressive results (77).

Passive Immunoprophylaxis

Passive immunoprophylaxis has been explored in solid organ transplantation in a number of randomised trials (78, 79, 80). However, only one trial in liver transplant recipients was placebo controlled (79). This study randomised 141 patients, a third of which received OKT3. The hyperimmune globulin provided significant overall protection from severe disease. However, no protection was seen in the D+/R- sub-group. The combined studies are difficult to interpret because of the different proportion of high-risk patients in each study group and

varying definitions of CMV disease. However, when the rates of CMV disease, as defined in each study, are compared in the treated and untreated groups, the results can be interpreted as demonstrating that intravenous immunoglobulin reduced the rate of CMV disease to approximately half of that seen in the placebo groups. Intravenous treatment is generally less convenient for the patient and health care provider, and carries the theoretical risk of transmitting blood-borne viruses (and vCJD in the UK). However, it does have the advantage of allowing compliance to be documented and on occasions this may have significant advantages. Availability of product limits the usefulness of this approach.

Anti-Viral Drug Therapy

Two approaches are in common use to minimise the impact of CMV infection or reactivation in solid organ transplantation: Universal Anti-CMV Prophylaxis, and Pre-Emptive Anti-CMV Therapy. In some units, both approaches are employed, depending upon the donor/recipient CMV status, organs transplanted, and severity of immunosuppression. The following sections summarise current evidence regarding their use.

Universal Anti-CMV Prophylaxis

In this approach, sub-groups of at risk patients are offered prophylactic antiviral therapy for an interval post transplantation, at doses designed to prevent disease. Therapy is usually offered to D+/R- combinations to prevent primary infection, less commonly to D+R+ combinations to minimise reactivation of latent virus and infection with new genotypes, and occasionally to D-/R+ combinations to prevent reactivation.

Treatment strategies have included the following:

Aciclovir

Aciclovir was shown to reduce CMV disease from 28% to 8% in a prospective placebo-controlled randomised clinical trial in renal transplantation (81). Others have shown no benefit in renal transplantation when ATG/OKT3 was used (82). In liver transplantation, the results have been contradictory with some or no benefit seen (83, 84). Aciclovir may therefore only provide significant protection in transplant recipients with lower risk profiles, such as D+R+ patients or where early immunosuppression is relatively modest e.g. in that small percentage of renal recipients who receive ciclosporin monotherapy. An alternative

strategy used CMV hyperimmune globulin in combination with aciclovir, but the utility of this approach is difficult to assess as the study was uncontrolled (85).

Valaciclovir

This pro-drug has a three to five fold improved oral bioavailability compared to aciclovir. Valaciclovir was studied in a randomised prospective placebo controlled study in a cohort of 208 D+/R- and 408 recipient positive renal transplant patients (38). In the high risk D+/R- group, the incidence of CMV disease at 90 days was 45% in the placebo group and 3% in the valaciclovir groups respectively - a highly statistically and clinically significant difference. There was also a significant difference in the seropositive recipients though the incidence of disease was low, 6% and 0%, depending on whether or not the donor was seropositive. After six months, the incidence of CMV disease had increased to 45% among seronegative recipients of the placebo and to 16% in the seronegative recipients of valaciclovir. There was also a highly significant statistical and clinical difference in the frequency of biopsy confirmed acute rejection with 26% of the valaciclovir group and the 52% of the placebo group in the D+/R- cohort experiencing rejection by six months.

Ganciclovir

A variety of regimens using relatively short courses of intravenous ganciclovir have been used in heart and renal transplant recipients (86, 87). In a randomised blinded study in renal transplant recipients, intravenous ganciclovir reduced the frequency of CMV disease to a quarter of that seen in the control group (88). Another randomised study compared intravenous ganciclovir with aciclovir, initially intravenous and then high dose orally, for 100 days in liver transplant recipients and found that CMV disease occurred in 0.8% of the immunosuppression ganciclovir and 10% of the aciclovir groups ($p=0.002$) (89). In contrast,

where the degree of immunosuppression more intense, as is typical in cardiac transplantation, 28 days of intravenous ganciclovir was shown in a placebo controlled randomised trial not to be effective in the highest risk (D+/R-) sub-group (86). In this situation other strategies need to be evaluated, such as the combination of antiviral drugs and CMV hyperimmune globulin or more protracted intravenous or oral drug therapy.

Despite the poor bioavailability of oral ganciclovir, a prospective double blind placebo-controlled randomised trial in liver transplant recipients (excluding donor and recipient seronegative patients) showed that the drug reduced morbidity due to CMV disease to about a quarter of that seen in the placebo group (38). These data are consistent with the findings of a similar study of 42 renal transplant patients randomised to receive either ganciclovir or aciclovir and followed for six months (90).

In a larger study, 155 D+/R- recipients of a variety of solid organs received 5-10 days of intravenous ganciclovir and then either oral ganciclovir or aciclovir for a further 12 weeks. Approximately one quarter also received antilymphocyte antibody therapy. There was no difference in the frequency of CMV 'syndrome' but tissue invasive CMV disease was seen in 10 out of 78 in the aciclovir and 3 out of 77 in the ganciclovir group (91). In a randomised prospective controlled trial of oral aciclovir vs oral ganciclovir in renal transplant recipients who received quadruple immunosuppression including OKT3, CMV disease occurred in the D+/R- subgroup in 5 of 13 receiving aciclovir and 0 of 14 receiving ganciclovir during the treatment phase. However, three patients in the ganciclovir group developed evidence of infection post-prophylaxis (92).

A retrospective study compared 60 renal transplant recipients who had received oral ganciclovir with 70 who had received valganciclovir. There was no difference in the incidence of CMV infection in the two groups, 6.9% vs 5.4% (93).

Valganciclovir

Oral valganciclovir gives plasma ganciclovir levels similar to those achieved with intravenous therapy and 10-fold higher than those achieved with the oral formulation of ganciclovir. This represents a significant advance for both treatment and prophylaxis (94). The key licensing data came from a randomised double-blind multi-centre study which recruited 364 adult CMV negative recipients of CMV positive solid organ transplants. The recipients were randomised 2:1 to valganciclovir or ganciclovir prophylaxis. The randomisation was stratified among the organ types with 177 liver, 120 kidney, 11 kidney/pancreas and 56 heart transplant recipients recruited. Treatment started within 10 days of transplantation and continued until 100 days post-surgery. The frequency of CMV disease in the first 6 months was 17.2% in the valganciclovir compared with 18.4% in the ganciclovir treated group. After 6 months, CMV disease occurred in 5% of the valganciclovir and 3.2% of the ganciclovir treated group (95, 96). The frequencies of detectable viral load and drug associated side effects were similar (97). At the end of the three month prophylactic period, 198 valganciclovir treated and 103 ganciclovir treated patients were assessed for the presence of ganciclovir resistant CMV strains. The incidence was low at 0% for the valganciclovir and 1.9% in patients who had received ganciclovir (98). Although the Federal Drug Authority did not license oral valganciclovir for prophylaxis in liver transplantation on the basis of these data, accumulating evidence means that most liver transplant units in the USA and UK now use valganciclovir prophylaxis.

A concern about a three month duration of prophylaxis is that of 'late onset disease', that is to say disease occurring after the period of prophylaxis. The frequency of late onset disease will crucially depend on the definition used for disease, and will also be influenced by the intensity of immunosuppression which the patients receive. As the time since the transplant lengthens, clinical monitoring becomes progressively less frequent and there is therefore a greater risk of delayed diagnosis of late (post-prophylaxis) CMV disease. In this pivotal study, CMV disease more than three months after transplantation was seen in about 18% of the group who had received prophylaxis.

A recent multicentre, double-blind, randomised controlled study compared 326 D+/R- renal transplant recipients randomised 1:1 to 200 days prophylaxis with valganciclovir versus 100 days prophylaxis followed by 100 days treatment with placebo (99). The rate of biopsy proven acute rejection was not significantly different, the rates of graft loss were low, and renal function was equal in the two groups. 97% of patients reported at least one adverse event but the distribution of these was equal across the two groups. The rate of other opportunist infections was significantly higher in the 100 day treatment arm, but this was almost entirely due to an increase in infections seen in the first 50 days. It is biologically implausible to ascribe this difference to drug therapy (or placebo) that was not due to start for another 50 days. Samples for measurement of CMV viraemia were collected at intervals (but the results not released to clinicians during the trial). The rate of CMV viraemia at twelve months was 51% in the 100 day arm and 37% in the 200 day treatment arm ($p < 0.05$). The total number of hospitalisations appeared to be similar in the two groups, but that due to CMV disease was reduced in the 200 day treatment group (10% vs 21%); however, it is not possible to calculate from the published data whether these differences were statistically significant.

The primary efficacy parameter was the proportion of patients who developed either 'CMV syndrome' or tissue invasive CMV within the first 52 weeks. 'CMV syndrome' was defined in this study as CMV viraemia with at least one of fever, malaise, leukopenia, thrombocytopenia or hepatitis. This definition of CMV syndrome as well as that of tissue invasive disease was congruent with that recommended by American authorities (100). In contrast to most studies, however, the authors of this study chose to define CMV disease as a combination of either tissue invasive CMV or CMV syndrome defined as above (99). Using this broadened definition of disease, 'CMV disease' was seen at one year in 16% of the 200 day treatment group versus 37% of the 100 day treatment group ($P < 0.0001$), of which 97.6% was in fact 'CMV syndrome'. Three patients experienced tissue invasive disease (all gastrointestinal), two in the 100 day treatment group and one in the 200 day treatment group (not significant).

When deciding whether doubling the length of prophylaxis with valganciclovir achieved a clinically useful risk-benefit ratio, one key assessment is how worthwhile it might be to approximately halve the rate of CMV disease, as defined by these authors. Of the CMV syndrome observed, which represented 97.6% of all CMV recorded, 45/59 cases in the 100 day group and 20/24 cases in the 200 day group were rated by the local investigator as of mild to moderate severity. The local clinicians decided to treat 57/59 of those with CMV syndrome in the 100 day group and all 24 patients with CMV syndrome in the 200 day group. This indicates that the local clinicians thought that the level of ill health justified treatment in these cases and implies (but does not prove) that they believed it was better to treat at this stage rather than wait until the patients' condition had potentially worsened.

Comparing 200 days of prophylaxis against 100 days, the number needed to treat to prevent one case of CMV disease (in practice, CMV syndrome) in the first year after transplantation was 5. A subsequent analysis showed that this benefit appeared to extend to at least two years after transplantation, indicating that the incidence of CMV disease (syndrome) was reduced, and not just delayed, by the longer period of prophylaxis (Humar et al, Transplantation). For comparison, in the first randomised placebo controlled study of oral ganciclovir in solid organ transplantation, the number needed to treat to prevent one case of disease in the D+/R- group of liver transplant recipients was approximately 3.5 (13).

The interpretation of this landmark study has varied across transplant centres in the UK, with some moving to a universal policy of 200 days of CMV prophylaxis in the D+/R- group, others continuing with a policy of 100 days of prophylaxis with a longer period for patients following treatment of rejection or at perceived higher infective risk. This may reflect the difficulty of extrapolating from a single study in which a non-standard definition of disease was adopted, together with concerns re the cost of additional therapy.

A study supported by the drug manufacturer examined the cost-effectiveness of extended therapy in the USA and indicated that such treatment reduced the incidence of CMV disease (syndrome) at a cost of \$15,000 per QALY (Blumberg et al, Transplantation). Such economic analyses are subject to many input variables and should be treated with caution, and the results may not be generalisable to other health care systems. However, the Scottish Medicines Consortium recently accepted that the *option* of extending valganciclovir prophylaxis to 200 days was probably cost effective in CMV D+/R- transplants (Ref http://www.scottishmedicines.org.uk/files/advice/valganciclovir_Valcyte_FINAL_DECEMBER_2010.doc_for_website.pdf, accessed 5th March 2011).

The recommended dose of valganciclovir is 900 mg once daily for patients with normal renal function, with reduction in dose at lower levels of GFR. A meta-analysis pre-published in e-format is of interest (Ref Kalil et al). This described the comparative use of 900 mg vs 450 mg of valganciclovir as prophylaxis against CMV in solid organ transplant recipients, with over 1500 patients analysed in each treatment limb. No difference was identified in the incidence of CMV using the lower dose of valganciclovir, but the 900 mg (recommended) dose was associated with an increased risk of leucopenia (odds ratio 3.32, $p < 0.0002$) and – surprisingly – an increased risk of rejection (odds ratio 2.56, $p < 0.005$). These risks persisted after adjustment for the type of allograft, CMV control strategy, and immunosuppression. The interpretation of these data is not clear, but a linked editorial (Avery, epub) recommends further study of the dose requirements for extended CMV prophylaxis, while noting the risk of an increased rate of anti-viral resistance when using the lower drug dose.

The evidence base for the use of oral valganciclovir prophylaxis is suboptimal and varies according to the organ transplanted. Where available, the data show equivalence for the use of oral valganciclovir and intravenous ganciclovir, but in many transplantation situations the absence of comparative trial data makes intravenous ganciclovir a reasonable preferred option. In practice, although current evidence supports the use of intravenous ganciclovir in simultaneous kidney-pancreas and cardiac transplantation, many units have begun to use oral valganciclovir prophylaxis to avoid the costs and inconvenience of hospital admission and/or home intravenous anti-viral therapy.

In a recent study involving eleven US centres, 136 lung transplants recipients who had completed 3 months of valganciclovir prophylaxis were randomly assigned to treatment with valganciclovir or placebo for an additional 9 months. CMV infection defined as disease or

positive viraemia or bronchoalveolar lavage culture but not meeting the primary end point occurred in 32% of short-course vs 4% of extended course treatment ($p < 0.001$), while CMV infection occurred in 64% vs 10%. There was no difference in other secondary endpoints which included acute rejection, opportunistic infection, ganciclovir resistance and safety (101). Because of the smaller number of thoracic transplants, the evidence base for prolonged CMV prophylaxis is more limited than for renal transplantation. In these guidelines, because of the high infection rates and the observation that the lung appears to be particularly badly affected by CMV infection, a recommendation has been made for prolonged anti-viral prophylaxis. More data would be welcome.

Pre-Emptive Anti-CMV Therapy

Prophylactic strategies have significant disadvantages. To be effective they rely to a greater or lesser degree on good patient compliance. The treatments add to the cost of the procedure and are unnecessary for a proportion of individuals who receive them. The agents have a side effect profile that must be balanced against the advantages of therapy. It is axiomatic that the larger the population exposed to a drug and the longer that exposure the higher will be the rate of drug resistance, assuming that the levels of drug achieved in vivo do not fully eradicate virus replication.

Because of the disadvantages of universal prophylaxis there has been interest in pre-emptive prophylactic strategies. In this approach, patients undergo regular surveillance and are treated when judged to be at high risk of developing CMV disease. Treatment is usually with valganciclovir now it is known that the kinetics of decrease in viral load is the same as for intravenous ganciclovir (102). A variety of markers for predicting future CMV disease have

been described such as the shell viral assay, PCR in serum, PCR from peripheral blood mononuclear cells, PCR from whole blood, and antigenaemia. Results should be interpreted in terms of the rapid dynamics of CMV replication as the average doubling time is 2.2 days (103). A publication which showed the plot of the probability of CMV disease against viral load in a renal transplant population is instructive (104). At a viral load of 5 log₁₀ copies per ml the probability was 20%, at 5.5 log₁₀ copies it was 50% and at 6 log₁₀ copies it was about 80%. Both of the molecular assays used predicted all cases of disease at a median time of about 12 days before the onset of symptoms. Others have used similar assays to reliably predict CMV disease and severity in kidney/pancreas (105) and other solid organ recipients (23, 106, 107).

The absolute levels of viraemia that are recommended as a threshold to start pre-emptive therapy will of course depend upon the assay used. For example, most real-time PCR assays (which are popular because of simplicity and high level of automation) report lower levels of viraemia compared to the Hybrid Capture Assay. Clearly the proportion of individuals who receive 'unnecessary' pre-emptive treatment vs those who develop CMV disease because they are not offered pre-emptive therapy will depend on where the threshold is set. Ideally units should establish the clinical significance of their local assay. The availability of a universal standard for molecular assays for CMV would facilitate cross-comparisons of viral load measurements from different laboratories and facilitate future multicentre trials without the explicit need for centralised testing.

That pre-emptive therapy can be used to control CMV disease has been demonstrated in many studies and in various meta-analyses. In a study of 52 asymptomatic renal transplant recipients, 23 (44%) had positive CMV PCR tests on at least one occasion. However, only

two (8.6%) developed CMV disease. This study suggests that, in this population with this assay, a treatment strategy based on positive PCR alone would treat a significant number of patients who did not necessarily require it. The authors reported the important finding that none of the 29 patients who were continuously negative for CMV PCR developed CMV disease (108).

Several authors have reported on a strategy that followed at risk patients with serial measures of CMV antigenaemia and then treated those predicted to be about to develop CMV disease with intravenous ganciclovir. In a study of 71 liver transplant recipients, CMV antigenaemia occurred in 22 and these patients were randomised into two groups. One received intravenous ganciclovir for seven days and the other oral ganciclovir for ten weeks. Although of low power because of the small sample size, it is striking that CMV disease was only seen in one patient, in the intravenous treatment arm (109). Two different strategies were adopted in a study of renal transplant recipients who were CMV seropositive at the time of transplantation. In one centre patients received oral ganciclovir for 12 weeks or until antigen negative for two consecutive weeks, and in the other centre intravenous ganciclovir for two weeks and then oral treatment until antigen negative for two consecutive weeks. Of 192 patients who met the study criteria, 90 were treated. All patients cleared antigen and there were no relapses. The single case of tissue invasive CMV disease occurred in the intravenous treatment group (110).

Of 49 patients who received unrelated donor bone marrow transplants and were either CMV seropositive or received a seropositive donor, 27 patients were enrolled in a pre-emptive strategy and 22 received prophylactic ganciclovir for four months. By one year the

probability of CMV disease occurring was 64% and 30% in the pre-emptive prophylactic and ganciclovir groups, respectively ($p=0.07$) (111).

A retrospective study of 39 renal, 28 liver and 23 heart transplant recipients noted 26 episodes of infection managed according to a pre-emptive strategy; 4 of these developed CMV disease but there were no deaths (112). However, there were also 21 episodes 'not managed according to the programme' where 12 individuals developed CMV disease and there were 2 deaths possibly related to CMV infection. This emphasises that it is most important that all the elements for appropriate monitoring are in place when a pre-emptive strategy is adopted.

A randomised trial in 69 liver transplant patients gave 8 weeks oral ganciclovir therapy or placebo when CMV DNA was detected by PCR (113). CMV disease developed in 12% of placebo recipients compared to 0% of those receiving ganciclovir. This trial provides the evidence base for using pre-emptive therapy to control CMV disease in recipients of liver transplants.

In a study in renal transplant recipients, 38 patients were evaluable from a group randomised to be monitored by CMVpp65 antigen tests. If a positive test was demonstrated the patients received oral ganciclovir. These were compared to a control group of 38 patients who received treatment if they developed disease. No patients in the pre-emptive group but nine in the control group developed CMV disease (114).

The duration of pre-emptive anti-viral therapy is important. The optimum length of treatment has not been determined, although some authors have recommended a minimum period of

four weeks. It is logical to be guided by serial measurements of the viral load and others have recommended treatment continue for two to four weeks after the patient has tested negative for viral replication (115). There is no trial evidence to inform the required frequency of viral load estimation, but expert opinion and the kinetics of viral replication suggest that such assessment should be performed at least weekly.

The authors of a review of prophylaxis strategies for CMV in solid organ transplantation commented on what has been termed ‘targeted prophylaxis’ in these guidelines and pointed out that ‘conventional prophylactic therapy has a large body of supportive controlled clinical studies demonstrating efficacy and cost-effectiveness. The strategy has the advantage of preventing other herpes viruses. There is some information to suggest that prophylactic therapy may benefit by reducing rejection’ (116). The authors contrast pre-emptive therapy, pointing out ‘it is limited by reliance on intensive surveillance with significant logistic difficulties and requiring good patient compliance. There is ambiguity about the best surveillance method and at the present time purported benefits of pre-emptive therapy, such as decreased cost, fewer adverse medication effects and less antiviral resistance have not been proven in head to head clinical studies’. In the counterpoint article published simultaneously, the problems of prophylaxis were discussed (117). These include preventing antigen presentation to the immune system so that patients are at risk of developing disease once the drug is stopped and the encouragement of drug resistance. More recent reviews have advocated targeted prophylaxis (118), the advantages of pre-emptive therapy (119), and the possibility of different approaches in each serological combination (120, 121).

One at least theoretical concern with a pre-emptive approach is that it would not be expected to protect from any indirect effects of CMV infection, including any influence on late graft

and patient survival (122). A recent randomised controlled study in kidney transplant patients has compared pre-emptive therapy to prophylaxis with oral ganciclovir (123). The data suggest that graft survival at 4 years post transplantation was significantly worse in patients who had been managed pre-emptively. More data are needed in this area but the suggestion is that prophylaxis interrupts initial organ amplification of CMV and hence minimizes effects on the graft, both in the early period post transplantation and also potentially in the longer term.

Both prophylaxis and pre-emptive therapy are effective at controlling CMV disease. Colleagues should discuss the practicalities with their local virologists and audit their agreed management strategy. At the time of writing the majority of UK units had adopted a targeted prophylaxis strategy.

Treatment of CMV Disease

Early references emphasise the need to reduce immunosuppression as well as giving specific antiviral therapy (124, 125). The former option is easier for renal rather than life-sustaining solid organ transplants where the risk of rejection may be considered prohibitive. Intravenous ganciclovir has had a major impact on the mortality and morbidity seen with this condition (125, 126, 127). Few would argue with the use of intravenous ganciclovir in this setting although the only randomised placebo controlled trial, in bone marrow transplant patients with CMV gastroenteritis did not show clinical benefit (128). In solid organ transplantation where there is fever and only trivial organ involvement, for example bone marrow involvement with a low white cell count, withdrawal of azathioprine or mycophenolate from a triple drug regime may be all that is required. If the patient is unwell (as opposed to merely uncomfortable) or there is evidence of organ dysfunction, most commonly with significant marrow suppression, hepatitis, gastrointestinal ulceration or pneumonitis, it is appropriate to reduce (by about half) the dose of calcineurin inhibitor and treat with intravenous ganciclovir.

Early concerns about neutropenia coincident with ganciclovir treatment have become less with greater experience and the appreciation that marrow suppression is often due to CMV disease and responds to anti-viral therapy. This approach to treatment is in line with that recommended by others (124, 129, 130).

Intravenous administration of ganciclovir remains the route of choice when the patient is seriously unwell when oral drug absorption is uncertain or poorly tolerated. However, it is now established that (oral) valganciclovir is of equal efficacy to intravenous ganciclovir for treating CMV disease in a mixed group of solid organ transplant recipients three quarters of

whom were renal transplant recipients (131). Substantial clinical experience in recent years has re-enforced that this is an appropriate treatment strategy, which has the advantage of being able to offer management as an outpatient for a proportion of patients.

Very high doses of intravenous hyperimmune globulin (0.5 g/kg body weight) have been used in conjunction with ganciclovir for the treatment of pneumonitis (132). It is not possible to evaluate the effectiveness of this treatment from the published literature, but the treatment is unlikely to have serious side effects and should be considered for life (or sight) threatening disease.

An important clinical point is the fact that infection with other co-pathogens is common in an immunosuppressed patient with CMV and other infections should always be ruled out by repeated clinical examination and special investigations. A decrease in the incidence of fungal infections was demonstrated in one sub-group of a randomised controlled trial of intravenous ganciclovir in cardiac transplant recipients (133).

The optimal duration of intravenous treatment after resolution of clinical signs is uncertain. Serial PCR of CMV DNA offers an objective measure of the degree of viraemia and may help to decide the duration of treatment. In practice, the clinical response as well as PCR measurements are used and, at least two weeks full dose treatment is recommended, with a longer duration of treatment if there is not a prompt fall in viral load (7).

There is a significant risk of relapse following successful treatment of CMV disease, with recurrent CMV disease reported in various organ recipients (134, 135, 136, 137, 138, 139). In one study in kidney and kidney/pancreas recipients, relapse was seen in approximately one

third of patients after treatment of the initial episode with ganciclovir (140). The quantitative measurement of CMV viral load may allow the risk of relapse to be predicted (141). Secondary prophylaxis after treatment of disease is usual practice, but deciding the duration of treatment is difficult. In general, prophylactic dose anti-viral therapy is usually prescribed for one month after resolution of CMV disease following mild illness, and for three months after severe illness, although there are no randomised trials to inform this strategy (7).

Drug Resistance

Although viral resistance has been relatively rarely reported in the transplant literature (142, 143, 144, 145), this may reflect under-reporting because of difficulties with the required cell culture assays. When PCR is used, 22% of 45 AIDS patients receiving long term ganciclovir develop resistance (146) and a figure of between 5 to 20% has been reported for solid organ transplant recipients (147, 148, 149, 150). The duration of therapy is likely to be important (151). Ganciclovir resistance has been demonstrated in about 8% of patients with AIDS after three months' treatment (152) rising to 11% with resistant blood or urine CMV isolates at six months, and 28% at nine months (153). The intensity of immunosuppression is likely to play a part in the frequency of ganciclovir resistance. This is illustrated by the report of six children with combined immunodeficiency who developed ganciclovir resistant CMV within ten days to three weeks after starting treatment (154). In a recent follow-up of the VICTOR study comparing valganciclovir and intravenous ganciclovir for the treatment of CMV disease, proven genotypic resistance was observed in 8 patients (~2.5%) despite the fact that these patients had high viral loads at the start of treatment, and a significant proportion failed to clear virus in plasma by day 21 of therapy. Interestingly this follow-up study also showed that the greatest risk for recurrent DNAemia was not drug resistance, but failure to clear virus from the plasma by day 21 (155).

In clinical practice, viral resistance is manifest by either progressive disease despite full dose anti-viral therapy, or a static or increasing viral load after drug treatment. Importantly, viral load is not a reliable indicator of drug resistance in the first weeks of treatment (156) since the natural kinetics of CMV replication prior to antiviral treatment means that patients with a rapidly increasing viral load are more likely to show a transient increase in viral load in the

first 5-7 days after therapy, before a decline is observed. This means that if the patient is recovering then increasing viral loads do not confirm a diagnosis of drug resistance.

Despite recent advances in technology, phenotypic assays are technically difficult, and have a slow turnaround time. Although they are important for setting reference standards, especially for novel mutations of unknown significance, they are not practical to guide clinical care. Genotypic assays can relatively rapidly detect gene mutations that are associated with both high and low grade resistance to ganciclovir as well as mutations that confer various degrees of resistance to ganciclovir, foscarnet and cidofovir. This is a rapidly evolving field with more laboratories having the capability to offer high throughput sequence based analysis of UL97 and UL54. Close liaison between transplant staff and local virological expertise is necessary to exclude the other reasons for non-response including patient non-compliance, prior to performing a relatively expensive drug resistance profile. For further reading a review is recommended (157).

Although there is no evidence beyond that of anecdotal clinical experience, the use of high dose hyperimmune CMV globulin is likely to be at least safe (158) and its effectiveness (if any) presumably uninfluenced by drug resistant mutations. However, it is not recommended as a single agent.

Cidofovir is a potential second line therapy unless there is a UL54 genotype that confers both ganciclovir and cidofovir resistance (159). It has significant side effects including nephrotoxicity.

Foscarnet is reserved as second or even third line therapy partly because of significant risks of nephrotoxicity and electrolyte disturbances, especially an acute reduction in ionised calcium. There is a smaller risk of neurotoxicity, particularly grand mal convulsions. However, there is extensive clinical experience of the agent, mostly for treating patients with AIDS. It remains a valuable option in the presence of virus resistant to ganciclovir (160, 161). In high risk clinical situations, consensus advice is to use combination treatment with ganciclovir and foscarnet (7). One trial in solid organ and stem cell transplant patients compared intravenous ganciclovir with a combination of half dose ganciclovir and half dose foscarnet and showed similar reductions in viral load when used as pre-emptive therapy, although more patients switched from the combination arm due to nephrotoxicity (162). Nevertheless, in patients where neutropenia is a problem, combination therapy may be appropriate.

There are novel anti-CMV agents in development, with maribavir and benzimidavir the most advanced (163, 164, 165). There is no cross resistance between maribavir and ganciclovir so this would be an attractive option in drug resistance.

The most recent pivotal phase III trials of maribavir, which targets the protein kinase activity of UL97, have yet to be formally published but have been disappointing despite encouraging phase II dose ranging/efficacy studies (166). The phase III trial for prophylaxis in haemopoietic stem cell transplant patients showed no difference in the incidence of DNAemia and pre-emptive treatment in patients receiving maribavir compared to those managed with pre-emptive ganciclovir therapy alone. The phase III study in solid organ transplant patients was terminated early since there appeared to be no significant benefit from maribavir prophylaxis. Given that this drug is highly potent at inhibiting replication of CMV in vitro,

these results are disappointing. The underlying reason for the failure of the drug in phase III studies is not fully resolved but may reflect the substantial serum protein binding of the drug and the dose that was selected for the phase III studies. Since maribavir targets the UL97 kinase which phosphorylates ganciclovir and aciclovir, it is not able to be used in combination with either of these drugs. However, all UL97 GCV resistant mutants tested to date have been susceptible to GCV. The maribavir resistant UL97 mutant at amino acid L397 is itself a poor protein kinase and so it is likely that maribavir resistant CMV strains may have a high fitness cost and be relatively ineffectual viruses (Emery VC and Shannon-Lowe C. Herpesviridae, in press).

The ability to restore CMV specific T cells to the patient would be highly attractive. There have been some research reports documenting the potential feasibility of transfer; however, this is likely at first to be only an option in haemopoietic transplantation (167).

There is some in-vitro evidence that both sirolimus and leflunomide have an anti-CMV effect. Published clinical anecdotes, which must be subject to publication bias, have indicated a beneficial effect of leflunomide in some cases (168, 169), but failure (in haemopoietic transplantation) in another case (170). In general, the mTOR inhibitors appear to be associated with a reduction in CMV infection/disease (171), although it is likely that this may be secondary to their effects on CMV specific T-cells rather than by a direct antiviral effect.

Suggested Audit Measures

The number of episodes of CMV disease diagnosed in the first year post transplantation should be collected and expressed as the number of episodes per transplant in the donor positive / recipient seronegative group and in the donor negative or positive and recipient seropositive group. This local data should be compared with current best practice as listed below.

CMV disease in solid organ recipients should be defined as an episode of ill health during which the patient experiences fever with another organ involvement such as bone marrow suppression, hepatitis, pneumonitis, transplant dysfunction, gastrointestinal tract involvement, or adrenalitis in a pattern typical for CMV induced dysfunction. Coincident with this it is necessary to demonstrate either typical histology, recovery of CMV from an affected organ, a diagnostic elevation in the quantity of circulating virus measured by molecular techniques, or a diagnostic rise in subsequent paired CMV IgG and IgM titres.

Current practice results in a CMV disease rate in the first year:

- of approximately 8% in D+/R- patients in renal transplantation
- of approximately 4% in D+/R- patients in liver transplantation
- of approximately 10% in D+/R- patients in kidney/pancreas transplantation
- approximately 15% in D+/R- patients in lung transplantation

- of approximately 10% in D+/R- patients in heart transplantation
- of approximately 15% in the donor seronegative or positive / recipient seropositive sub-group in lung transplantation

Statements of Potential Conflicts of Interest

Dr CG Newstead

I have received honoraria for lectures and teaching as well as expenses for travel and accommodation to attend scientific meetings from Fujisawa (now Astellas), Novartis, Roche and Wyeth (now Pfizer), most recently more than four years ago. I have received honoraria in the past for contributions to Advisory Boards for both Roche and Wyeth (Pfizer), but none for more than ten years. After election to the Council of the BTS and Chair of the Standards Committee more than ten years ago I declined invitations to contribute to Advisory Boards. My research and that of close collaborators has been in part sponsored by the above named companies as well as eight different Foundations.

Professor VC Emery

I have received honoraria for lectures and teaching as well as expenses for travel and accommodation to attend scientific meetings from Roche and Viropharma. I have received honoraria for participation in advisory boards for Elan Pharmaceuticals, Novartis, Roche, and Viropharma.

Dr PA Andrews

I have received travel and meeting expenses, but have not received lecture fees or contributed to Advisory Boards from Astellas, Novartis, Pfizer or Roche.

Dr JH Brown

I have received travel and meeting expenses from Amgen and Roche and participated in an advisory board for Wyeth.

Dr KJ Parameshwar

I have received honoraria for lectures and teaching as well as expenses for travel to attend scientific meetings from Novartis and Roche. I have also have received honoraria for participation in Advisory Boards for Roche.

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