CMV infection of transplant recipients

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CYTOMEGALOVIRUS (CMV) usually causes asymptomatic or clinically insignificant infection in the immunocompetent host. Thereafter, the virus persists in a latent state, but may be reactivated by immunosuppression.

Reactivation of latent infection most commonly occurs in the context of Human Immunodeficiency Virus (HIV) infection and allogeneic organ transplantation, but has also been described in other settings.

Chronic liver disease may be associated with reactivation of CMV. Tanaka et al. detected CMV DNA by polymerase chain reaction (PCR) in the blood of 77/122 (63.1%) patients with hepatic cirrhosis, but only 1/40 normal controls was PCR-positive (1). In the same study, PCR-positive patients with cirrhosis were antigenaemia assay-negative (the antigenaemia assay employs immunological methods to detect CMV-encoded antigens in peripheral blood smears). In cirrhotic patients, PCR-positivity was associated with worse liver dysfunction and with greater suppression of cellular immune responses.

Using PCR, CMV DNA has also been detected in the blood of 23/25 septic patients on an Intensive Care Unit (ICU), but not in controls (2).

Despite virological evidence of CMV reactivation in these patient groups (cirrhotic patients and septic patients), symptomatic infection did not develop.

This suggests that other factors are required for the development of CMV-associated disease. Donor virus is clearly the most important factor. Symptomatic infection of transplant recipients is usually associated with donor seropositivity, and is a consequence of the acquisition of donor CMV strain in the context of immunosuppression. When the recipient is seronegative, then primary infection occurs on a background of immunosuppression, and this infection is likely to be symptomatic.

In seropositive recipients, symptomatic infection is usually associated with excretion of the donor strain. The importance of donor CMV strain in determining the manifestations of CMV infection in transplant recipients was shown by Grundy et al. and by Chou. Of 74 renal transplant recipients in the study of Grundy et al., 51 were seropositive pre-transplant, and symptomatic infection only developed in those with seropositive donors (3). Consistent with this observation, CMV strains with identical restriction enzyme analyses have been isolated from organ recipients with a common donor (3,4).

Primary exposure to CMV does not, however, invariably result in infection. Chou examined nine pairs of seronegative transplant recipients who were exposed to nine seropositive donors. Four pairs seroconverted (and excreted virus), but five pairs remained uninfected (4). This observation supports the hypothesis that donor strain is the principal determinant of recipient infection, and also suggests that all seropositive donors are not equally infectious.

CMV infection in liver transplantation - incidence and risk factors

CMV infection has been reported in 30% to 50% of liver transplant recipients. In the same series, 12% to 25% of patients had symptomatic infection (also known as CMV disease) (refs. 5–13). A higher incidence of infection will be reported if prospective virological surveillance is undertaken. In Birmingham, the incidence of symptomatic infection is 12%. The incidence of CMV infection (including symptomatic and asymptomatic) in Birmingham recipients is 50%.

The likelihood of infection and disease depends on
the serological status of donor and recipient. Infection is principally dependent on organ donor seropositivity. When the donor is seropositive, then disease is more likely to develop in a seronegative recipient. The incidence of symptomatic infection in recipients with primary exposure ranges from 50% to 90% (11–13). The seronegative recipient of a liver from a seronegative donor has the lowest risk of symptomatic infection (13% to 22% in the same series).

Other risk factors for CMV infection and disease are detailed in Table 1.

Conventional diagnostic techniques

Viral culture

Human fibroblasts (MRC-5) cells are most commonly used for viral culture. Following inoculation, classic cytopathic effect is usually recognised within 2 to 3 weeks, but may take as long as 6 weeks to develop. This delay limits the application of conventional viral culture to clinical practice.

Early detection of CMV in cell culture can be achieved by modifications of the traditional technique. Such modifications include the use of immunofluorescence to identify viral antigens in cell culture. DEAFF (detection of early antigen fluorescent foci) detects CMV prior to development of classic cytopath. DEAFF and conventional viral culture appear to have equivalent sensitivities for CMV detection (14).

Centrifugal enhancement of viral culture is a relatively specific property of CMV. Centrifugation culture and immunohistology have been combined (in a technique known as “shell vial culture”) to confirm CMV infection at a very early stage. It has been suggested that shell vial culture is more sensitive for viral detection than conventional culture (15), but in other laboratories the converse has been found (16).

When viral culture is employed for CMV surveillance following transplantation, then blood and urine are most often examined. Isolation of CMV prior to the development of symptoms requires a rapid culture technique. Urine specimens are more frequently culture positive, but the predictive value of urine culture positivity (for the development of symptomatic infection) is inferior to that of blood culture positivity (17). Isolation of CMV from blood is strongly associated with symptomatic infection (8,16–19).

There is a tendency for virus to be isolated at an earlier stage from the specimens of symptomatic patients than from asymptomatic patients (16,17,20). Nevertheless, and despite the application of rapid culture techniques, many patients present with symptoms prior to the isolation of CMV from surveillance specimens (8,16,17,19,21–23).

These observations explain the poor sensitivity (for identification of potentially symptomatic patients at a presymptomatic stage) of those strategies which target pre-emptive therapy at asymptomatic culture positive patients (21–23).

Serology

Primary CMV infection may be associated with the development of an IgM antibody response. Reactivation of CMV (or infection by another CMV strain) in a seropositive recipient may be associated with an increase in IgG titre. Seropositive organ recipients frequently exhibit an IgM response in association with CMV reactivation or superinfection. The suitability of IgM assays for surveillance and diagnosis of CMV infection in transplant recipients has been addressed in a number of studies. The following characteristics of the IgM response have been described. Despite concurrent immunosuppression, an IgM response is observed in nearly all patients with primary CMV infection. An IgM response is observed in 26% to 55% of patients with reactivation or superinfection (24–27). An IgM response is observed in as many as 88% of patients with asymptomatic infection (16). The IgM response may be absent in patients with severe and overwhelming CMV infection (24) (28). IgM usually appears after the development of symptoms, and the interval from symptom onset to IgM positivity is characteristically 1 to 3 weeks (24) (29). IgM may appear earlier in reactivation infection than in primary infection (26). Viral culture positivity usually precedes the IgM response (assuming the application of rapid culture techniques). The timing of IgM appearance does not differ significantly between symptomatic and asymptomatic infection (24).

Alternative diagnostic techniques

Recognising the inadequacy of conventional virological techniques, two alternative diagnostic methods were introduced in the late 1980’s, and have been refined in the 1990’s.
The "antigenaemia assay" is now routinely used by many diagnostic laboratories for the management of transplant recipients.

The antigenaemia assay detects and quantitates CMV-infected leukocytes in the peripheral blood. Buffy-coat smears are examined, and CMV-infected cells are identified by immunoperoxidase or APAAP (alkaline phosphatase/anti-alkaline phosphatase) labelling after incubation with a mixture of CMV-specific monoclonal antibodies. More than 80% of antigen-positive cells are polymorphs. As the number of antigen-positive cells increases, the development of symptoms becomes more likely.

The results of the antigenaemia assay are generally concordant with the results of blood viral culture (30,31), though discordance may be observed during antiviral therapy (30,32).

The assay is highly sensitive and specific for the diagnosis of CMV infection (as defined by culture and serology), but specificity and positive predictive value for the diagnosis of CMV disease are less impressive. In other words, patients with asymptomatic infection (who do not subsequently develop symptoms) are frequently antigenaemia-positive.

Symptomatic infection is nearly always associated with a positive assay, and a negative assay effectively excludes CMV as the cause of symptoms (in evaluation of a pyrexial transplant patient). For this reason, the antigenaemia assay now has an established role for the confirmation of symptomatic CMV infection.

The antigenaemia assay is usually positive 1 to 2 weeks before a serological response is observed (31,33–36).

Unfortunately, the antigenaemia assay appears to have no role for surveillance of patients after transplantation. Surveillance specimens are frequently negative prior to the onset of symptoms, even when weekly monitoring is performed.

The antigenaemia assay cannot be recommended for routine surveillance of liver transplant recipients. Surveillance might, however, be appropriate in special circumstances e.g. monitoring the occasional patient who requires prolonged hospitalisation for management of septic complications.

PCR shares many of the assets and shortfalls of the antigenaemia assay. PCR is a sensitive and specific means of detecting viral nucleic acid, and has been applied to the detection of CMV DNA (and mRNA) in biological specimens.

CMV DNA has been detected by PCR in the blood of non-immunosuppressed seropositive blood donors (37,38). CMV DNA has also been detected in the blood of some ELISA-negative blood donors (50), suggesting that ELISA might fail to identify some latent infected individuals. The sensitivity of the PCR varies enormously, and is dependent on: (a) method of substrate preparation, (b) primer selection and constitution, (c) thermal cycling conditions including cycle number, and (d) post-PCR detection steps. Nested PCR is more sensitive than single-round PCR. For example, exquisite sensitivity will be achieved when nested PCR is combined with a post-PCR detection technique such as Southern blotting or ELISA. In these circumstances it is often possible to detect a single copy of target DNA. When an extremely sensitive PCR is used for CMV detection, then the technique will not differentiate immunosuppressed from non-immunosuppressed patients, and will not segregate immunosuppressed patients into non-infected, infected asymptomatic, and infected symptomatic groups. Therefore, the PCR assay required by the clinician managing transplant recipients must be relatively insensitive, and its ability to segregate these patient groups will be a direct function of that sensitivity. Disparate conclusions concerning the usefulness of PCR in this clinical setting reflect the application of assays with different detection sensitivities.

This concept assumes that the PCR has a detection threshold for any given set of substrates and conditions. It also assumes that the detection threshold is relatively constant, and therefore reproducible. Unfortunately the second assumption is untrue, and there is significant interassay variation in amplification efficiency. Problems of interpretation stemming from variable reaction efficiency can be partially overcome by the co-amplification of a known amount of standard DNA. An "internal" standard DNA may be used. The internal standard DNA sequence is nearly identical to that of the substrate target DNA, but differs in such a way (e.g. introduction of a restriction site or a short unique sequence) that amplified standard and amplified wild-type DNA can be differentiated in a post-PCR step. A known amount of internal standard DNA is added to each PCR reaction. Reduced PCR efficiency should result in a proportionate reduction of the two amplified species, so the ratio should be unchanged. This accounts for variable reaction efficiency, and most quantitative PCR assays are based on this principle.

Clinical studies of PCR in liver allograft recipients

For the majority of published studies, buffy-coat has been separated from blood, and used as substrate for the PCR reaction (29,39–44).

Plasma (29) and serum (42,44) have also been
used as substrate for the PCR. For detection of viral DNA in serum and plasma, nested PCR was used by Nyberg et al. (42), and by Schmidt et al. (29). Schmidt compared the results of serum nested amplification with buffy-coat single round amplification (for evaluation of the same blood specimen), and found 97% concordance. The detection of CMV DNA in serum clearly requires the use of a more sensitive PCR (such as nested PCR), or the addition of a post-PCR detection step such as hybridisation with a radiolabelled probe (44).

PCR is clearly more sensitive than viral culture, and culture-positive buffy coats are invariably PCR-positive (40,43,44). Schmidt et al. examined 264 specimens with both the antigenaemia assay and PCR, and found concordant results in 249 (94%) (29).

Buffy-coat PCR is nearly always positive at the time of symptom onset. Since false-negative results are not obtained at that time, sensitivity and negative predictive value of the PCR for the diagnosis of symptomatic infection are 100% (39,40,42). A positive result may be obtained up to 3 weeks prior to symptom onset (44). As assay sensitivity increases, then specificity and positive predictive value (for the diagnosis of symptomatic infection) decrease. In the study of Nyberg et al., most (24/25) patients had at least one PCR-positive buffy-coat (42). The specificity of the PCR (for symptomatic infection) in this study was only 8%.

Quantitation of CMV DNA by PCR

CMV DNA can be quantified by PCR including a competitive internal standard CMV DNA sequence (45-48).

We have applied a quantitative PCR assay to the examination of sequentially collected buffy-coat and serum specimens, and made the following observations (see Fig. 1). Viral titre rises slowly during the first 3 weeks after transplantation, then rises more quickly until the time of symptom onset. Titre then declines exponentially to approach early post-transplant titre 3 months after transplantation. Viral titre declines with resolution of symptoms. Titre declines more rapidly when specific antiviral therapy is used. There is a significant difference between peak viral titre observed for patients with symptomatic infection, patients with asymptomatic infection and patients with no evidence of CMV infection. High titre may be observed for patients with asymptomatic infection. Symptoms do not develop in patients with low viral titre. Serum titre mirrors buffy-coat titre.

This quantitative PCR assay is a useful research tool. It is likely that assays which quantitate blood CMV titre will soon be used in clinical practice.

Histology

When liver biochemistry is deranged in the setting of a febrile illness, then liver biopsy should be performed. The presence of CMV inclusions and/or positive immunostaining support the diagnosis of symptomatic CMV infection. During symptomatic infection with normal liver biochemistry, liver histology may be CMV-positive. In both settings (i.e. deranged and normal biochemistry), negative histology does not exclude the diagnosis of CMV infection. Hence, liver biopsy may provide a means for rapid confirmation of symptomatic CMV infection, but the sensitivity and negative predictive value of liver histology is uncertain (and would need to be established in a prospective study).

In conclusion, a range of diagnostic techniques are now available for use by the clinician to evaluate the transplant recipient. Some have good positive predictive value, and the more sensitive tests have excellent negative predictive value. It is often easier to exclude than it is to confirm symptomatic CMV infection. Some diagnostic tests are poorly standardised, so sensitivity and predictive value may vary from centre to centre. Rapid laboratory turn-around is essential. At present, rapid blood culture, the antigenaemia assay, or blood PCR are most suitable for assessment of febrile liver recipients. In the setting of liver dysfunction, liver biopsy may be preferable.
Prevention of CMV infection (strategies employed for liver transplant recipients)

Passive immunoprophylaxis with polyvalent or high-titre anti-CMV (hyperimmune) human immunoglobulin, Acyclovir, and Ganciclovir have been used to prevent CMV infection in liver transplant recipients. These agents have also been used in combination. Approaches to prophylaxis include:

- treatment of all recipients (excluding seronegative recipients of seronegative organs in some studies) from the time of transplantation,
- treatment of high-risk patients, such as those with primary CMV exposure (49-51) and those receiving OKT3 (52), and
- pre-emptive treatment targeted by surveillance cultures (22).

**Passive immunoprophylaxis**

Snydman et al. conducted a randomised, placebo-controlled, double-blind study of CMV hyperimmune globulin prophylaxis for liver recipients (10). In that study, treatment with hyperimmune globulin was associated with a reduced incidence (compared with placebo) of symptomatic CMV infection, and with fewer CMV-associated deaths (however, the magnitude of these beneficial effects did not achieve statistical significance). Prophylaxis was also associated with a statistically significant reduction in the incidence of CMV-associated fungal infection. Compared with other donor/recipient serological pairings, the study demonstrated little beneficial effect for seronegative recipients of seropositive organs.

Saliba et al. conducted a prospective, randomised, controlled study of CMV hyperimmune globulin prophylaxis for seronegative liver recipients (50). Prophylaxis was associated with a reduced incidence of CMV disease in recipients of seropositive organs (4/15), but primary exposure was associated with a very high incidence of symptomatic infection in the control group of this study (6/7).

Recently, the use of immune globulin to prevent symptomatic infection was examined by meta-analysis (53). The analysis concluded that:

- published results suggest that immune globulin reduces the incidence of symptomatic infection,
- a beneficial effect is observed in recipients with primary exposure, and
- hyperimmune products are not clearly superior to the polyvalent preparations.

Immunoglobulin has been used in combination with Acyclovir (51–56), and with Ganciclovir (56,57).

In conclusion, a reduction of symptomatic CMV infection can be achieved by passive immunoprophylaxis. The mechanism of protection is unclear, and may be independent of the titre of neutralising anti-CMV antibodies. The observed reduction of severe CMV-associated disease (principally CMV-associated fungal infection) is intriguing, but may be independent of the anti-CMV activity of the preparation.

**Acyclovir**

Compared with Herpes Simplex Virus (HSV), CMV is resistant to Acyclovir. CMV does not encode the thymidine kinase required for monophosphorylation of Acyclovir. Thus, acyclovir should not be used for treatment of established symptomatic infection, but the work of Balfour et al. suggested that high-dose Acyclovir (800 mg qid for patients with normal renal function) may provide effective prophylaxis against CMV in solid organ recipients (58).

Prospective analyses in liver recipients suggest that prophylaxis with high-dose Acyclovir is:

- more effective than the combination of short-term Ganciclovir and immunoglobulin (57).

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**TABLE 2**

The performance of diagnostic tests in assessment of the patient with symptomatic CMV infection (at the time of symptom onset)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>ppv</th>
<th>npv</th>
<th>Useful</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>++</td>
<td>++++</td>
<td>++</td>
<td>yes</td>
<td>&quot;rapid&quot; technique required</td>
</tr>
<tr>
<td>Urine</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>no</td>
<td>not recommended</td>
</tr>
<tr>
<td>Serology (IgM)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>no</td>
<td>not recommended</td>
</tr>
<tr>
<td>Antigenaemia</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>yes</td>
<td>commercially available</td>
</tr>
<tr>
<td>PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>++++</td>
<td>+</td>
<td>+++</td>
<td>possibly</td>
<td>to exclude CMV infection</td>
</tr>
<tr>
<td>Insensitive</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>yes</td>
<td>&quot;appropriate&quot; sensitivity</td>
</tr>
<tr>
<td>Liver histology</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>probably</td>
<td>always available, rapid results</td>
</tr>
</tbody>
</table>

ppv = a positive result confirms that symptoms are due to CMV infection.
npv = a negative result exonerates CMV as the cause of symptoms.
D. Mutimer

- less effective than prolonged Ganciclovir prophylaxis (59),
- inferior to a strategy based on pre-emptive Ganciclovir treatment of asymptomatic culture-positive recipients (22).

Retrospective analyses suggest that high-dose Acyclovir prophylaxis is:
- better than no prophylaxis (54,55),
- superior to low-dose Acyclovir (60), and
- inferior to sequential Ganciclovir/high-dose Acyclovir prophylaxis (56,60).

When combined with immunoglobulin, low-dose Acyclovir may decrease the incidence of symptomatic CMV infection in patients with primary CMV exposure (51).

Ganciclovir

Ganciclovir prophylaxis can prevent symptomatic CMV infection in liver transplant recipients (59). Winston et al. compared Ganciclovir with high-dose Acyclovir. Ganciclovir was given at a dose of 6 mg/kg, starting on the first post-transplant day, then daily until day 30. The same dose was then administered 5 times per week until day 100. CMV infection was observed in 6/124 Ganciclovir-treated patients (cf. 48/126 Acyclovir-treated patients), and only one patient developed symptomatic infection (cf. 12 Acyclovir-treated patients). Central venous access was required for Ganciclovir administration for the duration of prophylaxis. One assumes that this therapy was administered and monitored in outpatients and at home. The strategy is remarkable for the lack of morbidity associated with maintenance of venous access for this duration. In the United Kingdom, the cost of Ganciclovir required for this protocol is nearly £3,000 per 70-kg patient. The cost of administering the protocol is probably significantly greater.

Despite this convincing demonstration that symptomatic CMV infection is a preventable disease, few transplant groups are likely to adopt this protocol. Nevertheless, this study would have implications for CMV prophylaxis if oral Ganciclovir proves to be efficacious.

In a prospective, randomised study including seropositive recipients and recipients with seropositive donors, Cohen et al. failed to reduce the incidence of symptomatic infection by administration of prophylactic Ganciclovir during the third and fourth post-transplant weeks (61).

More selective prescription of intravenous Ganciclovir can reduce the incidence of symptomatic infection. In the only study of its type conducted in liver recipients, Singh et al. targeted asymptomatic patients with positive surveillance cultures for pre-emptive treatment with Ganciclovir (22). Consistent with the results of similar studies performed in bone marrow recipients (21,23), this approach lacks sensitivity for the presymptomatic identification of potentially symptomatic patients. The results of the study suggest that symptomatic infection can be reduced by approximately 50%, and that one patient receives unnecessary pre-emptive treatment for each patient correctly targeted (i.e. positive predictive value of a positive surveillance culture is approximately 50%). These remarkable results were achieved despite relatively infrequent surveillance sampling (only four samples in the first 8 post-transplant weeks) required by protocol. The strategy needs independent validation.

The potential of other surveillance techniques to target high-risk patients correctly should also be explored.

In conclusion, no prophylactic strategy can be recommended. The strategy adopted by a Unit should be commensurate with the incidence of symptomatic CMV infection observed in that Unit. For most Units, the incidence of serious CMV infection is decreasing. In Birmingham, the incidence of symptomatic infection is 12%, and pneumonitis affects fewer than 2% (and mechanical ventilation has not been required for many years). This low incidence of infection is observed without prophylaxis. The protocol of Winston et al. is clearly unsuitable for our programme.

The medical literature will be biased by the publication of studies with positive results. Strategies should be validated by controlled study before being adopted. Cost-benefit analysis should be an integral part of any strategy development.

Association of CMV infection with bacterial and fungal sepsis

An association of CMV infection with bacterial and fungal sepsis in renal (62) and cardiac (63) transplantation was recognised in the 1970's, before the widespread application of liver transplantation.

More recently, Smyth et al. confirmed this association of pulmonary bacterial infection with CMV infection and CMV pneumonitis in heart/lung recipients (64).

In an early report of the Pittsburgh experience, Singh et al. recognised the association of disseminated CMV infection with serious bacterial and fungal infection in patients who died after liver transplantation (13).

Bronsther et al. reported 17 cases of CMV hepatitis, and 8/17 died with bacterial and fungal sepsis (65).
George et al. analysed the results of Ganciclovir treatment of 17 liver recipients with CMV pneumonitis (66). In this series 11 patients died, and six died with fungal and bacterial sepsis. Four patients had hepatic artery thrombosis.

In a carefully performed prospective study, Paya et al. also found an association of hepatic artery thrombosis with CMV infection in liver transplant recipients (7).

Stratta et al. reported their experience with Ganciclovir treatment of CMV disease complicating liver transplantation (67). One hundred and three patients were treated, and 22 died. Seventeen of these died with bacterial and fungal sepsis.

Harbison et al. also reported their initial experience with Ganciclovir treatment of nine liver recipients (68). Six of nine patients had bacterial infection of the abdominal cavity and/or biliary tree. Three of nine patients had persistent CMV infection despite Ganciclovir treatment. Refractory CMV infection was associated with persistent abdominal abscess formation.

Mor et al. examined the results of high dose Acyclovir and immunoglobulin prophylaxis for liver recipients (54). CMV infection was associated with the deaths of seven patients in that study, and all seven died with invasive Candida infection.

CMV infection is clearly associated with serious bacterial and fungal infection, and this association merits further examination.

Does viral infection predispose to bacterial and fungal infection? Paya et al. undertook a multivariate time-dependent analysis of risk factors for bacterial and CMV infection (7). In multivariate analysis, asymptomatic CMV infection was a risk factor for bacterial infection, though the mechanism of this association was unclear.

In that study, risk factors for symptomatic CMV infection included thrombosis of the hepatic artery. Arterial thrombosis is almost invariably associated with severe and refractory bacterial biliary (intrahepatic and extrahepatic) sepsis.

Other studies suggest that bacterial sepsis precedes (and predisposes to) CMV infection. For instance, Harbison et al. described six patients with bacterial infection of the biliary tree and abdominal cavity preceding the onset of symptomatic CMV infection (68). Indeed, serious bacterial complications usually involve the biliary anastomosis, and frequently have an ischaemic basis. Biliary complications usually present in the first post-operative month, and symptomatic CMV infection usually develops in the second and third post-operative months. It is easier to reconcile these observations with the hypothesis that bacterial infection predisposes to CMV infection and not vice versa. Alternatively, bacterial and viral sepsis may be associated but not causally related.

At least two groups of investigators have suggested that bacterial infection predisposes to CMV infection, and that tumour necrosis factor alpha (TNF-alpha) may mediate that predisposition (69-71). Stein et al. used a transfected human monocyte cell line to demonstrate that TNF-alpha acts on an enhancer sequence to stimulate transcription of the CMV immediate early gene (72). The same investigators later showed an association of raised serum TNF-alpha with the subsequent development of CMV antigenaemia in solid organ transplant recipients (70).

Bacterial sepsis has also been associated with evidence of CMV replication in non-immunosuppressed patients (2).

**CMV infection in patients with fulminant hepatic failure undergoing liver transplantation**

A pre-transplant diagnosis of fulminant hepatic failure may also predispose liver recipients to the subsequent development of CMV infection. Paya et al. examined risk factors for CMV infection in a cohort of 79 consecutive liver recipients (7). A diagnosis of FHF emerged as a significant risk factor for CMV infection. That cohort included seven patients with FHF due to seronegative hepatitis, and 5/7 developed symptomatic infection.

In a preliminary report of Ganciclovir treatment for symptomatic CMV infection complicating liver transplantation, 4/9 patients were grafted for FHF (68). One of these recipients had CMV isolated from blood culture 2 days prior to transplantation. Stratta et al. observed an increased incidence of CMV infection in patients transplanted for FHF (11). The incidence of CMV infection in patients transplanted at their centre was 34.6%, but in patients grafted for FHF the incidence was 57.9% (11/19).

TNF-alpha might also mediate the association of FHF with CMV infection. Sheron et al. observed very high levels of TNF-alpha in the serum of patients with FHF (73). The highest levels of TNF-alpha were observed in patients who subsequently died (a group similar to those who would be considered candidates for liver transplantation).

An inverse correlation between TNF-alpha and interleukin-2 production by monocytes was also observed. IL-2 is required for clonal expansion of T and
B cells. Impaired immune responses to CMV infection might be observed in the setting of conditions associated with enhanced secretion of TNF-alpha.

**CMV infection and liver graft dysfunction**

The patient with symptomatic CMV infection is febrile, and disease may affect a single system (e.g., pneumonia or colitis). The liver may appear to be the principal focus of infection, and symptomatic infection with deranged liver function is usually labelled CMV hepatitis. Biochemical derangement is usually mild, and CMV hepatitis does not cause acute liver failure.

During symptomatic infection, liver biopsy frequently contains CMV inclusions and microabscesses, even when liver biochemistry is normal. In a patient with graft dysfunction, the presence of inclusions does not imply that dysfunction is due to CMV infection, and does not necessarily predict the subsequent development of symptomatic CMV infection.

Retrospective studies have suggested that donor/recipient HLA-DR matching is associated with an increased incidence of CMV hepatitis (74) and with the development of a form of chronic graft rejection known as the vanishing bile duct syndrome (VBDS) (74,75). CMV infection (defined by serology) (75) and primary CMV exposure (seropositive donor for seronegative recipient) (76) may also be associated with an increased incidence of VBDS. However, analysis of prospectively collected virological data by Paya et al. failed to confirm the association of CMV infection with HLA matching and development of VBDS (77).

The development of VBDS might be determined by a reduction of immunosuppression consequent upon the diagnosis of symptomatic CMV infection. Most physicians reduce immunosuppression in this setting, and azathioprine is frequently stopped in response to leukopenia. Candinas et al. observed an increased incidence of VBDS in patients who stopped azathioprine within the first 3 months (76).

**Conclusions**

CMV infection causes significant morbidity in a minority of patients. CMV rarely causes the death of liver transplant recipients. Instead, the patient who dies after liver transplantation usually succumbs to infection with multiple organisms (viral, bacterial and fungal) in the setting of multiple organ failure. Such patients frequently had poor preoperative condition, with advanced cirrhosis or fulminant hepatic failure.

Post-transplant CMV surveillance should only be undertaken if a positive result precipitates pre-emptive therapy. Despite the encouraging results of a single study, a role for routine surveillance and pre-emptive therapy remains unproven. Evaluation of a febrile patient requires the application of rapid diagnostic tests, such as the antigenaemia assay or PCR. These tests are sensitive, and have good negative predictive value. A negative result effectively excludes significant CMV infection. A positive result must be interpreted in the context of clinical features and other microbiology.

At present, antiviral prophylaxis should be reserved for high-risk patients, and probably requires the prolonged administration of Ganciclovir.

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