Drugs 1999 May; 57 (5): 735-741
0012-6667/99/0005-0735/$07.00/0

Cytomegalovirus Reactivation in Patients Infected with HIV

The Use of Polymerase Chain Reaction in Prediction and Management

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Abstract

Patients with HIV are living longer now than in the past, and with a better quality of life. During the advanced stages of HIV infection patients are at risk of cytomegalovirus (CMV) reactivation and subsequently CMV disease. It is important to review the evidence on whether CMV reactivation leads to CMV disease and what the best methods are for detecting such a reactivation. CMV polymerase chain reaction (PCR) can be used qualitatively to predict CMV disease and quantitatively to predict a general increase in mortality. CMV PCR can also be used to direct either prophylaxis or pre-emptive therapy to those most at risk of CMV disease. CMV PCR should be an integral part of the decision-making process when treating both new patients with CMV retinitis and those with disease reactivation.

1. The Relationship Between Cytomegalovirus (CMV) and HIV

Patients with advanced HIV infection, particularly when their CD4 cell counts fall below 50 cells/μl, are at risk of developing cytomegalovirus (CMV) disease. The majority of CMV disease manifests itself as retinal infection with gastrointestinal infection being the second most common manifestation. CMV disease of the central (encephalitis) and peripheral (polyradiculitis) nervous systems are becoming increasingly common particularly in patients receiving long term treatment for CMV retinitis. Once CMV disease is established the combination of induction and maintenance therapy will halt the progression of disease temporarily but relapse

Table I. Cytomegalovirus (CMV) reactivation detected by polymerase chain reaction (PCR) as a predictor of CMV disease

Study	No. of patients	CD4 count (cells/μl)	Follow-up (months)	CMV disease (%)	PPV (%)	NPV (%)
Bowen et al.[7]	97	<50	15	20	100	86
Shinkai et al.[8]	94	<100	18	27	96	92
Dodt et al.[9]	200	<100	15	19	95	85

NPV = negative predictive values; **PPV** = positive predictive values.

is inevitable. Therefore, the most effective strategy is to prevent CMV disease from occurring in the first instance by identifying those most at risk of disease and directing therapy to them. With the advent of molecular techniques such as the polymerase chain reaction (PCR), a better understanding has been achieved of the evolution of asymptomatic CMV infection to CMV disease and the importance of CMV viral load in disease pathogenesis. The aim of this paper is to review the role of CMV PCR in the diagnosis of CMV reactivation, CMV disease and its subsequent treatment.

Over 90% of patients with HIV are seropositive for CMV as defined by CMV-specific immunoglobulin G (IgG). As the CD4 count and, more importantly, the CD8 CMV-specific cytotoxic T cell count decrease, CMV reactivates. In patients with HIV the vast majority of CMV disease is caused by reactivation of an existing virus. However, primary infection can occur in patients who are seronegative for CMV. Therefore, care must be taken when administering blood products, and advice on safe sexual practices should be given. In order to prevent CMV disease in HIV infected hosts, markers are needed that can identify patients most at risk of reactivating CMV and thus, developing future disease. Early studies used conventional cell culture to detect CMV reactivation in patients with advanced HIV infection, but results were disappointing showing positive predictive values (PPV) between 0.35 and 0.50.[1,2] Subsequently, molecular techniques such as the polymerase chain reaction (PCR) and antigenaemia have been shown to be successful at identifying patients with AIDS and both established CMV retinitis and non-retinal CMV disease.[3-6]

2. Prospective Studies

There have now been 3 large prospective studies of patients with advanced HIV infection designed to elucidate the relationship between the reactivation of CMV and the development of CMV disease. These 3 important studies are summarised in table I showing patient demographics, follow-up, incidence of CMV disease and positive and negative predictive values (PPV, NPV) for each type of assay used. The relative hazards for the detection of CMV disease by positive results with these assays are shown in table II.

In a prospective study, 97 patients were followed monthly and underwent whole blood PCR for CMV DNA.[7] The results showed that the detection of CMV by PCR at baseline was significantly associated with the development of CMV disease (fig. 1).[7] CMV PCR positivity in these patients conferred a significantly greater relative hazard for the development of CMV disease than CD4 count (relative hazards 20.15 and 0.90, respectively). Shinkai et al.[8] reported similar results in 94 patients (every 3 months, plasma PCR and cell culture) where the relative hazard for CMV disease in the PCR-positive group was 23 for plasma PCR compared with 9.2 for viraemia and for 2.3 viruria.

The third study compared CMV plasma PCR with pp65 antigenaemia and cell culture in 200 patients in relation to the development of CMV disease.[9] Dodt et al.[9] again not only showed that PCR was superior to antigenaemia and cell culture in the prediction of CMV disease, but also found that PCR became positive (lead time) at a median of 46 days prior to CMV disease compared with 34 days for antigenaemia and 1 day for cell culture (table II). In a fourth study just published, Walmsley et al.[10] found the lead time for DNA hybrid capture was

152 days compared with 167 days for antigenaemia on monthly blood samples. In their paper the authors offer a direct comparison of all DNA and antigenaemia primers/products used in different studies and their sensitivities/specificities for CMV disease.

3. Practical Recommendations

Patients in the advanced stages of HIV infection should therefore, be regularly monitored for evidence of CMV replication in the blood using qualitative CMV PCR where possible or antigenaemia if PCR is not available. Lead times from PCR positivty and detectable antigenaemia range from 46 to 180 days and 34 to 176 days, respectively. Therefore, monitoring patients every 2 to 3 months should be sufficient. In our centre, patients with CD4 counts <100 cells/litre are monitored with CMV PCR every 8 weeks. Patients who are PCR-positive are then followed monthly with quantitative PCR and full ophthalmological assessment. The negative predictive value of all the above assays is near 100% so PCR-negative patients are followed with PCRs only every 2 months.

4. Quantitative Relationship Between CMV DNA and Disease

Further work has looked at the absolute quantity of CMV DNA in relation to disease development. We found that the median viral load was significantly higher in those patients who went on to develop disease (4.77 *vs* 4.0 $\log_{10}$ copies/ml whole blood, p = 0.02).[7] CMV load continued to increase over time and each 0.25 $\log_{10}$ increase in viral load was significantly associated with a 37% increase in the likelihood of developing CMV disease (relative hazard 1.37; 95% CI: 1.15 to 1.63, p = 0.0004). This effect was unchanged after adjusting for the age and CD4 count of patients. Shinkai et al.[8] also found that CMV disease development was proportional to peak plasma copy numbers of CMV. All patients with CMV loads >1000 copies/μl plasma (4.0 $\log_{10}$) developed disease compared with only 33% of those with <100 copies/μl plasma.

5. Prognostic Significance

Apart from the morbidity associated with the development of CMV disease, CMV PCR is a prognostic indicator for increased mortality. In a prospective study of 45 patients with AIDS and CMV retinitis, those patients with high CMV loads in blood at presentation of retinitis (>4.95 $\log_{10}$) were less likely to respond virologically to induction therapy and had a shorter time to first progression of retinitis.[11] Importantly, these patients with high viral loads had a significantly reduced survival of 5 months compared with those patients with CMV DNA <4.95 $\log_{10}$.[11] These findings were confirmed in a virological substudy of a large randomised placebo-controlled study of oral ganciclovir *vs* placebo. This study looked at CMV PCR positivity in plasma in both treatment and placebo arms in relation to both CMV disease and overall survival.[12] In the placebo arm, patients who were PCR-positive at baseline had a 2.5-fold increased risk of death, independent of CD4 count. Baseline plasma CMV load was also a significant variable for survival where patients with a baseline viral load of >50 000 copies/μl plasma had a median survival of 400 days compared with 600 days for patients with baseline loads of <2500 copies/μl

Table II. Relative hazards and time to cytomegalovirus (CMV) disease for each assay

Study	PCR	pp65 (antigenaemia)	Viraemia	Viruria
Bowen et al.[7]	20			
Shinkai et al.[8]	23		9.2	2.3
Dodt et al.[9]	30	22	20	
Time to disease[9] (days)	46	34	1	

PCR = polymerase chain reaction.

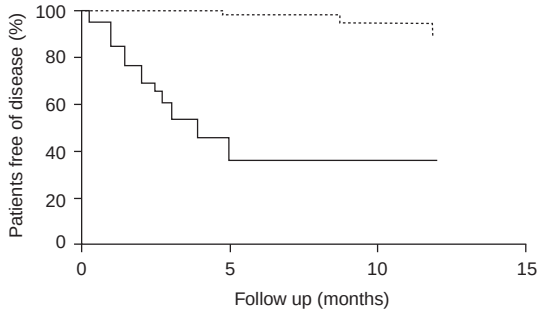

Fig. 1. Kaplan-Meier analysis of time to cytomegalovirus (CMV) disease according to baseline polymerase chain reaction (PCR) status. The patients who were PCR-positive (solid line) were significantly more likely to progress to CMV disease than those who were PCR-negative (dotted line) [$p < 0.0001$]. Reproduced from Bowen et al.,[7] with permission.

plasma. In patients receiving placebo, each $\log_{10}$ increase in baseline viral load was associated with a 2.2-fold increase in mortality.[12]

All the above studies support the role of PCR to identify patients most at risk of developing CMV disease and highlights the important prognostic significance of a high CMV load where quantitative PCR is available. Commercially available quantitative kits are currently being evaluated against standard PCR assays for this purpose.[13]

6. Treatment

Based on the results in the previous section, it is clear that treatment should be commenced before CMV viral load has escalated (or at first qualitative PCR positivity) and disease has ensued. However, questions remain over when drug therapy should be introduced and what drugs should be used? In patients with low CD4 counts but no evidence of CMV replication empirical treatment with anti-CMV agents is termed 'prophylaxis'. Targeting therapy to high-risk patients who have evidence of systemic CMV reactivation as detailed above but no evidence of end-organ CMV disease has been termed 'pre-emptive therapy'.[12] The term 'treatment' is then reserved for patients with CMV disease. Pre-emptive therapy is not a new concept and has been successfully employed in the prevention of CMV disease in transplant patients for several years.[14-16] There have not yet been any randomised controlled clinical studies of pre-emptive therapy for CMV retinitis in patients who are HIV-positive.

Two large studies have investigated the use of ganciclovir and valaciclovir, respectively, as 'oral prophylaxis' in patients with advanced HIV infection (CD4 <100 cells/μl) and no evidence of CMV disease.[17,18] Spector et al.[17] in a large double-blind randomised controlled study of oral ganciclovir *vs* placebo found that oral ganciclovir was effective in reducing the prevalence of CMV disease from 30% in the placebo arm to 16% in the ganciclovir arm. Feinberg et al.[18] demonstrated that valaciclovir, the valine ester prodrug of aciclovir, reduced CMV disease from 18% in the placebo group (received conventional aciclovir at either 800mg once daily or 400mg twice daily) to 12% in the treatment arm. Both these studies, despite being known as prophylactic studies, actually comprised a mixture of patients who were CMV-negative receiving true prophylaxis and patients who were CMV PCR-positive receiving pre-emptive therapy.

The virology substudy of the ganciclovir trial found that patients who were PCR-negative on entering the trial received the greatest benefit from ganciclovir. Of the patients who were PCR-positive at baseline only those with CMV loads <50 000 copies/μl plasma received any benefit from the drug.[17] This suggests that of the patients at low risk of CMV disease (i.e. PCR-negative) some were unlikely to develop disease (74% of patients randomised to placebo did not develop CMV disease) and remained PCR-negative. In the patients who became PCR-positive with low viral loads during the study, oral ganciclovir was sufficient to prevent early subclinical CMV replication. However, once CMV replication was well established the poor oral bioavailability of oral ganciclovir and low plasma and intracellular concentrations of the drug were unable to arrest CMV replication.

In contrast, in a subset of patients recruited to the AIDS Clinical Trials Group 204 (ACTG 204) virology substudy, valaciclovir was more effective

in reducing CMV disease in patients who were CMV PCR-positive in blood at baseline (i.e. those receiving valaciclovir pre-emptive therapy) than in those patients who were CMV PCR-negative at baseline.[19] This contrasting result to the oral ganciclovir study may be a reflection of the greater oral bioavailability of valaciclovir or the more efficient intracellular triphosphorylation. However, this was a substudy of a small number of patients and further studies on larger numbers would be needed to confirm and elucidate the mechanism for this finding.

In order to further study the efficacy of oral ganciclovir in pre-emptive therapy, we performed a small pilot study on 20 patients who were CMV PCR-positive and randomised to receive oral ganciclovir either 3 or 6 g/day for 28 days.[20] The 6 g/day dosage was more effective than 3 g/day at reducing high baseline CMV viral loads to undetectable levels and maintaining this effect after treatment was stopped. It is known that the majority of patients with CMV disease will be CMV PCR-negative after 14 days of intravenous ganciclovir and all patients will be PCR-negative at 21 days.[11] Therefore, further studies comparing intravenous ganciclovir with the 6g dose of oral ganciclovir as pre-emptive therapy are warranted.

6.1 Highly Active Antiretroviral Therapy

All the studies mentioned above, including the study of the natural history of CMV infection in the HIV infected host, were performed prior to the routine use of protease inhibitors or highly active antiretroviral therapy (HAART).[21] The incidence of CMV disease has fallen sharply since the introduction of HAART and this has made it difficult to recruit patients to any new studies of pre-emptive CMV therapy.[22] Recently, HAART itself has been shown to decrease CMV viral load. 16 patients who were CMV PCR-positive (median CMV load 5 $\log_{10}$) became PCR-negative between 5 to 40 weeks following the introduction of a protease inhibitor to their antiretroviral regimen without the use of any specific anti-CMV therapy.[23] Over 14 months of follow-up, 14 of 16 patients have remained PCR-negative and there have been no cases of CMV disease. Two patients who became PCR-positive as a result of interruptions to their HAART became PCR-negative again after alterations to their antiretroviral regimen.[23] Ongoing studies at the Royal Free Hospital and as part of ACTG 360

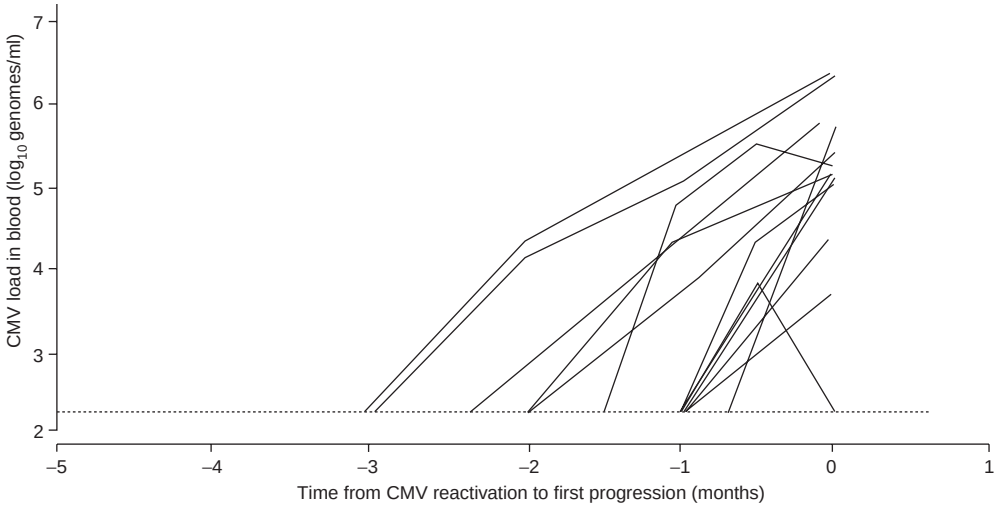

Fig. 2. Cytomegalovirus (CMV) load measurements in individual patients during maintenance therapy preceding the first progression of CMV retinitis (reproduced from Bowen et al.,[24] with permission).

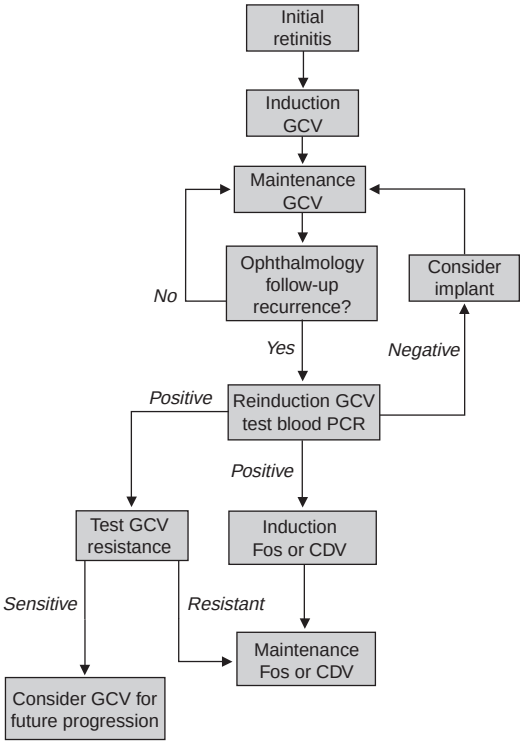

Fig. 3. Suggested flow diagram for the management of patients presenting with cytomegalovirus (CMV) retinitis incorporating the use of CMV polymerase chain reaction (PCR) [reproduced from Bowen et al.,[24] with permission]. **CDV** = cidofovir; **Fos** = foscarnet; **GCV** = ganciclovir.

may further define the changing natural history of CMV reactivation and disease in the era of HAART.

7. Role of Polymerase Chain Reaction in Treatment Monitoring

CMV PCR can also be used to monitor patients receiving CMV maintenance therapy and optimise their treatment. The majority of patients have a first recurrence of their retinitis without any evidence of systemic CMV replication. Such local reactivation of retinal disease is a result of suboptimal vitreal concentrations of antiviral chemotherapy. This implies that regular ophthalmological follow-up remains the most sensitive method for detecting progression of retinitis. However, in one study all patients who became CMV PCR-positive in blood had a progression of their retinitis and were more likely to develop CMV disease elsewhere.[24] Moreover, most of these patients had been PCR-positive for approximately 1 month prior to progression and CMV load increased prior to progression (fig. 2). Therefore, in these patients, particularly if they are receiving oral ganciclovir maintenance therapy, a 'pre-emptive' course of high dose re-induction intravenous therapy might delay retinitis progression.

PCR monitoring could also be used to identify patients who are PCR-negative with a low risk of systemic disease who could be managed with topical therapy alone (such as intravitreal injections or intra-ocular implants). Following an episode of retinitis progression very few patients who were PCR-positive became PCR-negative after intravenous re-induction therapy. In most patients who become PCR-positive during ganciclovir maintenance therapy UL97 mutations conferring ganciclovir drug resistance can be detected.[24] Therefore, in patients who become PCR-positive a change of antiviral therapy could be indicated. The use of CMV PCR in the management of patients with CMV retinitis can be seen in figure 3.

8. Conclusion

CMV reactivation signifies that patients with HIV are at a high risk not only of CMV disease but also of increased mortality. CMV PCR is a sensitive 'surrogate marker' for CMV disease and can be used to identify those patients most at risk of CMV reactivation and subsequent CMV disease. CMV load is a further important indicator not only of disease pathogenesis (with an increased CMV morbidity and overall mortality), but also of likely response to treatment (both pre-emptive and induction therapy). Despite treatment, CMV load has a significant bearing on mortality and asymptomatic patients who are CMV PCR-positive should be treated to reduce CMV load and prevent CMV disease. Specific anti-CMV therapy can be targeted to those with evidence of reactivation but currently

available oral treatments are not effective against high CMV loads. The monitoring of CMV by PCR could be effectively used to identify patients for new trials of pre-emptive therapy. The introduction of HAART has made the biggest impact not only on end-organ CMV disease but also on asymptomatic CMV reactivation and should, therefore, be a vital part of the treatment of patients with advanced HIV infection and CMV reactivation.

Acknowledgements

I would like to thank my colleagues at the Royal Free Hospital, particularly Professor PD Griffiths and Dr VC Emery for all their help and support. I would also like to thank Dr Jane Deayton for her comments and discussions related to CMV and HAART.

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