Cytomegalovirus DNAemia and disease: incidence, natural history and management in settings other than allogeneic stem cell transplantation

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Background and Objectives. Despite increasing intensity and profound immunosuppression associated with newer therapies for hematologic malignancies, little information exists regarding cytomegalovirus (CMV) reactivation in settings other than allogeneic stem cell transplantation (SCT).

Design and Methods. We reviewed the epidemiology of CMV disease in patients who were CMV polymerase chain reaction (PCR) positive during treatment for hematologic malignancies without allogeneic SCT from June 1999 to June 2004.

Results. Thirty-six patients with CMV reactivation were identified. Of these, 92% were undergoing investigation for fever. Fifteen patients with CMV DNAemia were treated with ganciclovir without CMV disease developing. Notably, 20 patients with untreated CMV DNAemia did not develop CMV disease during a median follow-up of 3.5 (1-19) months. The highest rates of reactivation were observed with HyperCVAD (7.8%) and alemtuzumab (50%).

Interpretation and Conclusions. We recommend that screening for CMV DNAemia be instituted and pre-emptive therapy contemplated for asymptomatic CMV reactivation only in patients receiving alemtuzumab therapy, but not routinely for other patients outside the allogeneic SCT setting. Indeed for such patients, detection of isolated CMV DNAemia does not imply the need for immediate therapy and future studies are needed to validate PCR detection of CMV DNA and CMV DNA titers as predictors for CMV disease.

Key words: cytomegalovirus, CMV DNAemia, CMV disease, ganciclovir.

Haematologica 2005; 90:1672-1679

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Cytomegalovirus (CMV) reactivation or primary infection occurs in 15 - 80% of recipients of allogeneic stem cell transplants[1] and is associated with significant morbidity and mortality.[2] Well-defined strategies have therefore been developed for prophylaxis, screening and pre-emptive treatment,[1,3-5] using sensitive diagnostic testing for CMV such as pp65 antigenemia and CMV DNAemia.[3, 6-10] The risk of CMV disease occurring with other therapies for hematologic malignancies had been thought to be very low[11-13] but again with potentially significant CMV-related morbidity and mortality.[11] However, there has been a rising frequency of CMV disease noted with the increasing use of more potently immunosuppressive therapies,[11,14,15] necessitating a review of the incidence and natural history of CMV disease in this population. The aims of this study were: (i) to determine the epidemiology of CMV infection; (ii) to identify subgroups at risk of CMV infection; (iii) to identify potential relationships between CMV DNAemia and CMV disease, and; (iv) to describe treatment outcomes of CMV infection within a population of hematology patients not undergoing allogeneic transplantation.

Design and Methods

A retrospective case series of patients with hematologic malignancies undergoing treatment at the Peter MacCallum Cancer Centre (PMCC) between 1 June 1999 and 31 June 2004 was performed. The PMCC is a tertiary cancer institution that treats a broad spectrum of malignant conditions. Using a laboratory database, all patients treated at PMCC with a positive CMV polymerase chain reaction (PCR) result from tissue, bronchoalveolar lavage fluid, serum and blood specimens were identified. Of 56 patients, those who were CMV PCR positive on urine, mucosal or skin swab samples only, without CMV DNA detected in whole blood, were excluded. Patients who had undergone any form of

allogeneic stem-cell transplantation prior to the CMV PCR result were also excluded. All diagnostic and screening tests for CMV were performed by a single reference laboratory, the Victorian Infectious Diseases Reference Laboratory (VIDRL) during the study period. A qualitative *herpes viridae* multiplex PCR assay in which primers for CMV, *Herpes simplex* virus type 1, *Herpes simplex* virus type 2 and *Varicella zoster* virus are used in each PCR reaction and the presence of specific viral DNA identified from a positive PCR product was used to detect CMV DNAemia, and has been described elsewhere.[16] Quantitative determination of the CMV DNA viral load in CMV DNAemia positive samples was undertaken at the discretion of the VIDRL and not performed on all samples. The number of patients who had undergone *herpes viridae* multiplex PCR testing at the VIDRL was also determined. These patients were assumed to have suspected *herpes viridae* infection at the time of investigation, unless identified by chart review to have undergone pre-emptive screening.

Pharmacy records at the PMCC were used to identify patients who had been treated with ganciclovir during the study period, and pathology database records were reviewed to identify patients with histological evidence of CMV disease. Chart review was performed to identify baseline CMV serological status and potential risk factors for CMV reactivation, including the number of previous therapies, the type of therapy prior to CMV reactivation, and underlying hematologic disease. Indications for PCR testing and information regarding therapy, clinical outcome and follow-up were also obtained. Therapy immediately prior to CMV infection was classified as *conventional chemotherapy*, fludarabine-containing regimens, autologous stem cell transplantation (SCT), denileukin diftitox (recombinant interleukin-2 conjugated to diphtheria toxin) and monoclonal antibody therapy with rituximab or alemtuzumab. Denileukin diftitox and alemtuzumab were primarily used in patients with relapsed or refractory disease. Rituximab was used in conjunction with conventional-dose chemotherapy regimens. All patients undergoing autologous SCT received unmanipulated stem cells. Allogeneic transplants were not performed at the PMCC. The total number of patients treated with each of these approaches during the study period was determined and used to estimate the event rate of CMV reactivation or primary infection within each subgroup. This was defined as the number of patients identified with CMV DNAemia and/or CMV disease divided by the total number of patients treated with a given therapy over the period of this study. The number of patients within each subgroup who had undergone investigation with *herpes viridae* multiplex PCR was also determined and these patients were assumed to have suspected *herpes viridae*-related illness at the time of testing. The policy for transfusion of blood products remained consistent throughout the study period. CMV negative recipients were administered blood products from donors serologically negative for CMV. CMV serology was determined at the VIDRL using commercial ELISA kits (DiaSorin ETI-CYTOK-M reverse PLUS and ETI-CYTOK-G PLUS). Additional blood leukoreduction filters were not routinely used for red cell or platelet products during this period. No routine CMV anti-viral prophylaxis was given. *Herpes simplex* virus prophylaxis was valacyclovir 500mg daily or acyclovir 200mg b.i.d. for autologous SCT and HyperCVAD regimens[17] until the absolute neutrophil count exceeded 1.0×10^9/L.

Definitions

We used previously published definitions of CMV infection.[18] In brief, CMV reactivation was defined as the isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen. CMV DNAemia was defined as the isolation of CMV DNA in whole blood, plasma, or peripheral blood leukocytes by a PCR-based technique. CMV disease was defined as clinical symptoms or radiological evidence consistent with CMV end organ infection together with histopathological or immunohistochemical features of CMV infection on tissue biopsy specimens. In the presence of a new or changing pulmonary infiltrate, detection of CMV DNA in broncho-alveolar lavage fluid was considered supportive evidence of CMV pneumonitis if it was otherwise unsafe to obtain a tissue specimen.[19]

Anti-CMV therapy

During the study period, CMV disease was treated with ganciclovir 5 mg/kg IV b.i.d. adjusted for renal function. At the discretion of the treating physician, ganciclovir was also used to treat illness suspected to be related to CMV infection in selected patients with CMV DNAemia, or asymptomatic CMV DNAemia with high or rising CMV DNA titers. Patients with CMV DNAemia diagnosed at the time of a concurrent bacterial or fungal cause for fever, spontaneously defervesced on anti-bacterial or anti-fungal therapy or who remained asymptomatic without rising CMV titers while monitored for CMV DNAemia while receiving alemtuzumab, were observed without anti-CMV therapy at the discretion of the treating physician.

Statistical analysis

A descriptive analysis was performed. Fisher's exact test was used to assess differences between categorical variables. Stata Version 7.0 was used for statistical analysis [StataCorp, 1997 #1150].

Results

Patients' characteristics

Of 2331 patients treated at the PMCC between 1999 and 2004, 36 patients fulfilled the study inclusion criteria. These patients were aged between 35-77 years (median 58.5 years), and 18 (50%) were male. Patients had received a median of two therapies (0-9) prior to the treatment during which CMV reactivation was detected, with 64% having received two or more prior therapies.

The underlying diagnoses were acute leukemia (n=6), chronic lymphocytic leukemia (n=6), B-cell non-Hodgkin's lymphoma (n=13), peripheral T-cell lymphoma (n=4), mycosis fungoides (n=3), γδ T-cell lymphoma (n=1), Hodgkin's lymphoma (n=1) and multiple myeloma (n=2). The high proportion of patients with lymphoma in our cohort is a reflection of the patient population treated at the PMCC.

Baseline CMV serological status and anti-viral prophylaxis

Thirty-one patients had positive baseline CMV IgG serology prior to the time of CMV infection. Four patients were IgG negative, and baseline CMV serology was not performed in one patient. Twenty-eight patients (72%) were receiving anti-*herpes simplex* virus prophylaxis concurrently with therapy immediately prior to the diagnosis of CMV reactivation: 26 with valacyclovir and two with acyclovir.

Clinical findings at the time of investigation

Of the total 36 patients, 35 patients had CMV DNAemia on whole blood and one patient was diagnosed with CMV disease on tissue biopsy but without CMV DNAemia at the time of investigation (Table 1). In patients with CMV reactivation, the major clinical indication for *herpes viridae* multiplex PCR testing was persistent fever for which no bacterial or fungal cause had been identified at the time of investigation (92%). Chest X-ray abnormalities were reported in 25%, and abnormalities in liver function tests were reported in 3%. Three of twelve asymptomatic patients receiving alemtuzumab underwent prospective screening for CMV reactivation. All developed CMV DNAemia, with two remaining asymptomatic: one treated with pre-emptive ganciclovir and one with close observation until CMV DNA negativity.

Herpes viridae multiplex PCR testing and CMV reactivation

One hundred and seventy patients with hematologic malignancies from the PMCC who had undergone *herpes viridae* multiplex PCR testing on whole blood between 1999-2004 were identified. The number of tests performed each year increased linearly over this time period. Of patients with a positive *herpes viridae* multiplex PCR, only eight patients were identified with *herpes viridae* DNA other than CMV [*Herpes simplex* virus (n=7), *Varicella zoster* virus (n=1)] (Table 2). The event rate for CMV reactivation was determined for several clinical parameters, including the type of therapy administered immediately prior to CMV infection. Alemtuzumab therapy was associated with an overall 50% event rate of CMV reactivation. This was higher than the event rate among patients receiving HyperCVAD (9.7%), denileukin diftitox (6.1%), autologous SCT (4.2%), fludarabine-containing regimens (4.6%) or rituximab (2.6%). Patients receiving other conventional-dose chemotherapy regimens had a less than one percent event rate of CMV reactivation.

Table 1. Clinical findings at the time of investigation.

Clinical Status	CMV DNAemia (N=35)	CMV Disease (N=7)	Total
Fever	33	–	94%
Persisent fever alone	11	–	31%
Chest X-ray abnormality	10	3	28%
Gastrointestinal symptoms	4	4	11%
Skin rash	2	–	6%
Liver function abnormality	1	–	3%
Asymptomatic	2	–	6%

Table 2. Therapy at the time of CMV reactivation.

Primary treatment type	Total number of patients treated	Number tested for herpes viridae DNAemia N (%)	Number with positive CMV DNAemia N (%)
Conventional chemotherapy	1636	61 (3.7%)	13 (21.0%)
HyperCVAD	62	15 (24.2%)	6 (40.0%)
Fludarabine	151	31 (20.5%)	7 (22.6%)
Autologous SCT	191	34 (17.8%)	8 (23.5%)
Denileukin diftitox	33	6 (18.2%)	2 (33.0%)
Rituximab	225	17 (7.5%)	6 (35.3%)
Alemtuzumab	12	6 (50.0%)*	6 (100.0%)

**3 patients (25%) underwent herpes viridae multiplex PCR for suspected CMV-related illness and 3 asymptomatic patients (25%) underwent pre-emptive screening for CMV DNAemia while receiving alemtuzumab.*

Table 3. Details of patients with proven CMV disease.

Case	Number of previous therapies	Therapy prior to disease	Disease	Biopsy	CMV DNAemia	CMV titer (copies/mL)	Steroid therapy	Outcome (death on day after diagnosis of CMV disease)
53/F	3	Autologous SCT	Colitis	Positive	Multiple	<400	No	
72/M	1	Autologous SCT	Colitis	Positive	Multiple	107869	No	Died (60)
63/F	4	Autologous SCT	Pneumonitis*/Esophagitis	Positive	Multiple	29793	Yes	
67/F	4	Autologous SCT	Gastritis/Esophagitis	Positive	Negative	Not tested	Yes	
64/F	3	Fludarabine rituximab	Pneumonitis*		Multiple	11900	Yes	Died (150)
35/F	4	Denileukin diftitox	Pneumonitis*		Single (tested once)	145534	Yes	Died (14)
59/M	2	Denileukin diftitox	Pneumonitis*		Multiple	75262	No	

Pneumonitis was diagnosed on CMV DNA detected by herpes viridae multiplex PCR on broncho-alveolar lavage specimens.

The proportion of patients undergoing investigation with *herpes viridae* multiplex PCR for each therapeutic subgroup is given in Table 2. This proportion was lowest amongst patients receiving conventional chemotherapy (3.7%) and rituximab (7.5%). In patients receiving therapies other than alemtuzumab, the proportion of patients tested was 18.2-24.2%. In patients tested for CMV reactivation, 21-40% were positive across the treatment groups. In contrast, all patients receiving alemtuzumab who underwent testing for symptomatic illness or as pre-emptive screening developed CMV reactivation (Table 2).

CMV disease

Seven patients were diagnosed with CMV disease (Table 3). Six of these patients had CMV DNAemia detected by PCR assay at the time of diagnosis, whereas one patient with histologically proven disease did not have concurrent CMV DNAemia. Of note, of all patients with CMV reactivation, 5/10 (50.0%) patients who were not receiving anti-*Herpes simplex* virus prophylaxis at the time of CMV reactivation developed CMV disease compared to 2/26 (7.7%) patients who were receiving prophylaxis (p=0.01, Fisher's exact test).

Gastrointestinal disease

Four patients who developed histologically proven CMV gastrointestinal disease and in all four this occurred following autologous SCT. Symptoms of disease developed a median of 45 days (29-70 days) after the day of stem cell infusion, and a median of 32.5 days (20-35 days) following neutrophil recovery. All patients had developed significant mucosal toxicity during their autologous SCT.

Pneumonitis

Three patients developed pneumonitis with new radiological infiltrates on chest X-rays or chest computed tomography scans, despite conventional antibacterial therapy. These infiltrates were associated with CMV DNA positivity on broncho-alveolar lavage specimens. Two patients developed pneumonitis 26 and 36 days after commencement of treatment with denileukin diftitox, and one patient 19 days following fludarabine, cyclophosphamide and rituximab therapy. Pneumonia or pulmonary edema from other causes preceded or was concurrent with the development of pneumonitis; however, CMV pneumonitis was suspected due to clinical deterioration or a progressive radiological infiltrate.

Four patients were observed to develop CMV disease, two with gastrointestinal disease and two with pneumonitis, while receiving high-dose corticosteroids. These patients were significantly pre-treated, having received a median of four prior therapies.

CMV DNAemia

Of the 35 patients who were identified with CMV DNAemia, six patients had concurrent CMV disease, eight patients without proven CMV disease but with fever and symptoms not explained by other causes were treated presumptively for symptomatic CMV reactivation, nineteen patients were diagnosed with a non-CMV related illness at the time and two asymptomatic patients were receiving alemtuzumab and undergoing pre-emptive screening.

Treatment and outcomes

Sixteen patients were treated with ganciclovir, seven patients with proven CMV disease, eight patients without proven CMV disease but with fever and symptoms not explained by other causes and one patient with asymptomatic CMV reactivation with rising CMV DNA titers while receiving alemtuzumab. Twenty patients with CMV DNA in blood who were not treated with ganciclovir did not develop symptoms of CMV infection or end organ disease over a median

follow-up period of 3.5 months (range 1-19 months), including one asymptomatic patient receiving alemtuzumab therapy who was monitored until resolution of CMV DNAemia. No patients died from CMV disease (Table 4).

Table 4. Outcomes of patients with CMV DNAemia or disease.

Outcome	*Intravenous ganciclovir given*	
	Yes	*No*
	N=16	*N=20*
Final CMV Status		
CMV disease	7	0
Suspected CMV infection	8	0
Symptomatic non-CMV illness with CMV DNAemia	0	19
Asymptomatic CMV DNAemia	1	1
Death	5	6
Progressive hematologic disease	1	4
Bacterial pneumonia	0	1
Post-operative respiratory failure	1	0
Pulmonary embolism	1	0
Cardiac arrest	1	0
Toxic megacolon	1	0
Hemophagocytic syndrome	0	1

Discussion

Although the mechanisms, risk factors and time course underlying CMV reactivation and disease are well described in allogeneic SCT, this is not the case in other settings. More potent T-cell immunosuppressive therapies are increasingly recognized to be associated with significant opportunistic infections,[20-22] including CMV disease.[14,15,23-25] Simultaneously, methods to detect CMV reactivation have been refined from relatively insensitive viral cultures, shell vial techniques, and direct fluorescence antigen testing, to more sensitive techniques detecting the pp65 CMV viral protein or CMV DNA.[8] CMV reactivation has consequently been observed to be more common than previously noted,[15,26-29] but does not always lead to the development of CMV disease.[8,15,26,27,29]

CMV infection: possible mechanisms

CMV is a member of the *herpes viridae* family. Most CMV infections occur as reactivation of latent virus from a myeloid and mononuclear cell reservoir in previously infected patients.[1,30] Several factors can play a role in CMV reactivation. *In vitro*, inflammatory cytokines, including interleukin (IL)-1β, IL-4 and tumor-necrosis factor (TNF) stimulate CMV gene expression via major regulatory proteins IE-1 and IE-2 acting on early CMV viral promoters.[31] Cell-mediated immunity with CMV-specific $CD8^+$ and $CD4^+$ T lymphocytes[30,32-35] are important in preventing CMV infection. Significant and sustained decreases in absolute $CD8^+$ and $CD4^+$ T lymphocyte numbers occur following autologous SCT,[36,37] as well as after treatment with the purine analog, fludarabine,[20] and alemtuzumab.[14,23] Conversely, the ability to reconstitute CMV-specific $CD8^+$ T cells has been observed to be a protective factor against CMV infection following autologous SCT.[38] Alemtuzumab has also been associated with depletion of other $CD52^+$ cell types, including dendritic cells[30,39] and NK cells[30,40] which may impair host immune surveillance for controlling viral replication.

CMV DNAemia in settings other than allogeneic SCT

In previously published series, autologous SCT recipients were noted to have a 2–9% incidence of CMV pneumonitis but a case-related mortality comparable to that in allogeneic SCT recipients of 80%.[41-44] Similarly, an autopsy-based, single center, retrospective study of patients with acute leukemia, chronic leukemia or myelodysplasia not treated with allogeneic transplantation, reported a low, but rising prevalence of CMV pneumonitis over a 5-year period, with a case fatality rate of 57%.[11] Immunosuppressive therapy, including fludarabine, high-dose cyclophosphamide and steroids and granulocyte infusions from unscreened donors were proposed as risk factors. There is one published case report on CMV reactivation following the use of rituximab in post-transplantation lymphoproliferative disorder[45] but no previous reports of CMV reactivation following therapy with denileukin diftitox. More recent studies have reported a significant incidence of CMV reactivation associated with the use of the anti-CD52 antibody, alemtuzumab,[14,46,47] and this aspect has recently been reviewed.[48] Although prospective studies have examined the incidence of CMV DNAemia by PCR in the setting of autologous SCT[28] and therapy with alemtuzumab,[46] no prospective studies have validated whether the detection of CMV DNA in peripheral blood by the PCR methodology is predictive for symptomatic CMV disease in the non-allogeneic SCT setting.

The current study is significant in demonstrating that, despite the use of immunosuppressive therapies with autologous SCT, fludarabine-based therapy or biological therapy with rituximab or denileukin diftitox, the event rate of CMV reactivation was relatively low, at less than 10%. It was noted that 18.2-24.2% of patients receiving HyperCVAD, fludarabine, autologous SCT, or denileukin diftitox were investigated for *herpes viridae* reactivation whereas only 3.7% of those receiving conventional chemotherapy and 7.5% of those treated with rituximab were investigated in this way. This is likely a reflection of the greater hematologic and/or immunosuppression of these therapies leading to persistent fevers for which no definitive bacterial or fungal cause was identified. Patients who underwent autologous SCT had not received total body irradiation (TBI) conditioning regimens which are recognized as more

immunosuppressive than non-TBI-based regimens.[49] Although investigation of only symptomatic patients receiving these therapies is likely to have underestimated the true incidence of CMV reactivation, it has been previously noted that the majority of patients with asymptomatic CMV reactivation do not develop disease.[8,15,27-29] Our findings would therefore support a strategy of investigation of symptomatic disease[12] in patients receiving all therapies examined here with the exception of alemtuzumab, as screening asymptomatic patients is unlikely to be cost effective.

CMV DNAemia and alemtuzumab

Conversely, patients receiving alemtuzumab had an event rate of CMV reactivation of 50%, including all three symptomatic patients screened for *herpes viridae* reactivation and all three asymptomatic patients undergoing pre-emptive screening for CMV reactivation. This is in keeping with the findings of a recent prospective study.[46] Early trials with alemtuzumab had also noted a high incidence of symptomatic CMV reactivation of up to 18%,[24,25] albeit in significantly pretreated populations.[14,24,25,40,50] Although the underlying disease may be postulated as contributing to the risk of CMV reactivation, a recent review noted that for patients receiving alemtuzumab, the risk of CMV reactivation was not higher in patients with cutaneous T-cell lymphomas than in patients treated for chronic lymphocytic lymphoma.[48] Recent guidelines have subsequently recommended screening for asymptomatic patients receiving alemtuzumab.[47,48,51] The high event rate of CMV reactivation in this study would support such a strategy, although the risk of developing CMV disease in this population is unknown.

CMV disease

Despite the small number of patients in our study with CMV disease, there were several noteworthy observations regarding the natural history and potential risk factors. Symptoms of CMV disease occurred at a median of 36 days after commencement of therapy, which is earlier than historical data for allogeneic SCT recipients who had not undergone screening and pre-emptive therapy.[2] CMV disease occurred in four out seven patients who had received high-dose corticosteroids in addition to other therapies. Corticosteroid use is a known risk factor for CMV disease following autologous SCT.[15,29] It was interesting to note that CMV disease also occurred in sites that had experienced significant tissue inflammation: gastrointestinal disease occurred in patients with mucosal toxicity from autologous SCT and pneumonitis occurred in patients with other prior or co-existent pulmonary disease. This observation highlights the potential difficulty in differentiating treatment-related toxicity from CMV disease, and suggests that a high clinical index of suspicion for CMV infection is required. Additionally, CMV DNA in blood did not invariably accompany CMV disease, although six out of seven patients with disease had a high CMV DNA titer of >5,000 copies/mL or positive CMV DNA on multiple occasions.

Concurrent prophylaxis against *Herpes simplex* virus also appeared to protect against the development of CMV disease, although used at doses considered subtherapeutic for CMV infection.

CMV disease and CMV DNAemia

The use of PCR-based detection of CMV DNA in blood to predict CMV disease has not been prospectively validated in settings outside allogeneic SCT, particularly those settings in which the incidence of CMV disease is low.[8] As with other published data,[1,15,26,28,29] detection of CMV DNA by PCR in our study identified a significant number of patients who did not develop disease, suggesting that detection of CMV DNA in blood has a low positive predictive value for CMV disease.

The CMV DNA viral load in blood may be more informative in predicting patients at risk of developing CMV disease[28,52,53] and quantitative PCR viral load thresholds of >5,000-10,000 copies/mL of CMV DNA[52,53] have been proposed to be predictive of symptomatic CMV disease.[53] The predictive value of such thresholds or rising CMV DNA titers does, however, require methodological standardization,[54] and prospective validation for a variety of diseases and therapies before adoption. Caution must be exercised when interpreting such investigations depending on the clinical setting, as symptomatic CMV disease can develop before CMV DNA is detected, depending on the timing and frequency of investigation for reactivation.[5,8]

Recommendations for monitoring and anti-CMV therapy

Several recommendations can be made regarding CMV reactivation for patients receiving therapy in the non-allogeneic SCT setting. In patients receiving alemtuzumab, weekly monitoring for CMV DNA with PCR is recommended given the high reactivation rates.[47,48,51] Despite previous recommendations for pre-emptive therapy with parenteral ganciclovir for asymptomatic CMV DNAemia in this cohort,[47,48] an optimal management strategy remains to be determined. Our study and others have shown that patients with asymptomatic CMV DNAemia may not develop CMV disease, despite not receiving anti-CMV therapy; indeed, one of the six patients in our study who received alemtuzumab did not develop CMV disease. In asymptomatic patients with CMV DNA detected in blood, parenteral ganciclovir is not clearly indicated and may have potentially adverse consequences such as myelosuppression,[5] creating a possible predisposition to invasive

fungal infection,[26,28] and introducing the risk of late CMV reactivation due to impaired development of CMV specific immunity.[3,5] Close observation and monitoring of CMV DNA positivity in blood until negativity can be considered. Treatment can be instituted for patients who become symptomatic or develop high or rising CMV DNA titers.[48,51] In patients who are treated, parenteral ganciclovir is effective and has been recommended in published guidelines.[47] Oral ganciclovir has also been used successfully in one small study[46] for asymptomatic CMV reactivation, but valganciclovir[55] with better oral bioavailability should be evaluated.

Treatment with parenteral ganciclovir in patients with proven CMV disease is clearly indicated. In patients with an acute illness potentially related to CMV reactivation, empiric commencement of ganciclovir may be clinically indicated pending further investigation for CMV reactivation. Investigations using PCR to detect CMV DNA in blood should be considered in such symptomatic patients in whom bacterial, fungal and other causes are unlikely. Detection of CMV DNA in blood in this setting may be supportive, especially if there is a high CMV viral load >5,000-10,000 copies/mL, but not definitive and clinical correlation with pursuit of histological diagnosis, when feasible, should be undertaken. Conversely, a negative result for CMV DNAemia does not exclude CMV disease, and treatment should be commenced if histologic findings demonstrate CMV infection. Further studies are required to determine the true positive- and negative-predictive value of PCR methodologies for the detection of CMV DNA.

Further study

Despite higher rates of detectable CMV reactivation being observed as more potent immunosuppressive therapies are used and more sensitive diagnostic techniques are implemented, the natural history of CMV reactivation and predictive factors for CMV disease in non-allogeneic SCT settings remain largely unknown. Our study has identified areas in which further investigation is required, including determination of the predictive value of CMV DNA in blood in various treatment settings, and the identification and validation of other predictive factors for the development of CMV disease. These may include clinical criteria as well as other immunological measures. Recently, the use of major histocompatibility complex tetramers to monitor CMV-specific $CD8^+$ cytotoxic T lymphocytes in order to predict patients at risk of CMV infection and disease has shown promise in this regard.[56] Such information is critical for making treatment decisions, as CMV disease still carries a significant morbidity and mortality in non-allogeneic SCT settings.

AN and LW are responsible for the whole work, including the conception, design, and conduction of the study, analysis and interpretation of the data and drafting and revising the manuscript; AN, LW, LC, KT were involved in acquisition of the data; AN, LW, KT were responsible for data analysis; AN, LW interpreted the results and drafted the manuscript; all authors were involved in the discussion and revision of the manuscript and gave their permission for the final version submitted for publication. The authors declare that they have no potential conflict of interest.

We acknowledge the contribution of Julian Druce, Jennie Leydon and Jack Richards at the Victorian Infectious Diseases Reference Laboratory for providing laboratory data.

Manuscript received April 30, 2005. Accepted September 21, 2005.

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