JOURNAL OF CLINICAL MICROBIOLOGY, May 1998, p. 1333–1337 Vol. 36, No. 5
0095-1137/98/$04.00+0

Evaluation of Murex CMV DNA Hybrid Capture Assay for Detection and Quantitation of Cytomegalovirus Infection in Patients following Allogeneic Stem Cell Transplantation

HOLGER HEBART,[1]* DAPHNE GAMER,[1] JUERGEN LOEFFLER,[1] CLAUDIA MUELLER,[1] CHRISTIAN SINZGER,[2] GERHARD JAHN,[2] PETER BADER,[3] THOMAS KLINGEBIEL,[3] LOTHAR KANZ,[1] AND HERMANN EINSELE[1]

Medizinische Klinik und Poliklinik, Abteilung II und Sektion für Transplantationsimmunologie und Immunhämatologie,[1] Institut für Epidemiologie und Klinik der Viruskrankheiten,[2] and Universitätskinderklinik, Abteilung Kinderheilkunde 1,[3] 72076 Tübingen, Germany

Received 2 October 1997/Returned for modification 2 December 1997/Accepted 26 January 1998

Murex hybrid capture DNA assay (HCS) is a solution hybridization antibody capture assay for detection and quantitation of cytomegalovirus (CMV) DNA in leukocytes. To determine whether CMV HCS is sensitive enough to initiate and monitor antiviral therapy after allogeneic stem cell transplantation (SCT), 51 consecutive SCT recipients were prospectively screened for the appearance of CMV infection by HCS, PCR, and culture assays from blood samples. Preemptive antiviral therapy was initiated after the second positive PCR result in all patients, as previously reported, and HCS was not considered for clinical decision making. A total of 417 samples were analyzed. Of these, 21 samples were found to be positive by PCR and HCS, 88 samples were PCR positive but HCS negative, and 308 were negative by both assays. Concordance of results between PCR and HCS and between HCS and blood culture was observed in 78.9 and 95.9% of the samples assayed, respectively. PCR was found to be more sensitive than HCS, and HCS was more sensitive than the blood culture assay ($P < 0.0001$). Four patients with symptomatic CMV infection were PCR positive prior to the onset of CMV-related symptoms, whereas HCS detected CMV DNA in three patients prior to and one at onset of CMV disease. The numbers of genomes per milliliter of blood were higher in patients with symptomatic CMV infection than in those with asymptomatic CMV infection ($P = 0.06$). None of the HCS-negative patients developed CMV disease. Thus, all patients with CMV disease were correctly identified by HCS; however, the lower sensitivity limit of the HCS assay may still be insufficient to allow diagnosis of CMV infection early enough to prevent CMV disease in patients following allogeneic SCT.

In spite of recent developments in diagnosis and treatment, cytomegalovirus (CMV) infection remains a major infectious complication in recipients of an allogeneic stem cell transplantation (SCT) (15). Symptomatic CMV infection, especially when leading to interstitial pneumonia, is still associated with a high mortality rate in these patients (6, 16). Early diagnosis of CMV infection and preemptive antiviral therapy, based on the PCR technique, resulted in a significant reduction of the incidence of CMV disease and of CMV-associated mortality following allogeneic SCT (3–5). Thus, pp65 antigen detection in leukocytes (1), and PCR analysis of leukocytes and plasma (3–5, 19) are widely used for the early diagnosis of CMV infection.

However, the various protocols applied are poorly standardized (9). To make test results in international drug trials comparable, the standardization of the diagnostic assays applied is mandatory. Murex hybrid capture CMV DNA assay (HCS) is a commercially available solution hybridization antibody capture assay for the quantitative detection of CMV DNA in leukocytes. HCS allows early diagnosis and monitoring of antiviral therapy in AIDS patients and in solid-organ-transplant recipients (11, 13, 14, 18). To determine whether HCS is sensitive enough to initiate and to monitor antiviral therapy after allogeneic SCT, HCS was compared to PCR with whole blood and to rapid and conventional virus culture techniques for the detection and quantitation of CMV in blood samples.

* Corresponding author. Mailing address: Medizinische Klinik und Poliklinik, Abteilung II, Otfried-Müller-Str.10, D-72076 Tübingen, Germany. Phone: 0049 7071 2982712. Fax: 0049 7071 293179. E-mail: holger.hebart@uni-tuebingen.de.

MATERIALS AND METHODS

Patients. Between December 1994 and February 1996, 51 consecutive patients after allogeneic bone marrow transplantation ($n = 46$) or allogeneic peripheral blood SCT ($n = 5$) (median age, 33 years; range, 2 to 57 years) were included in the study. Conditioning therapy consisted either of busulfan (four doses of 1 mg/kg of body weight on days −7 to −4) and cyclophosphamide (two doses of 60 mg/kg of body weight on days −3 and −2) or of total body irradiation (two doses of 2 Gy on days −6 to −4) and cyclophosphamide (two doses of 60 mg/kg of body weight, days −3 and −2). Patient characteristics at the time of transplant are shown in Table 1. Oral acyclovir was administered in all cases at a dosage of 400 mg four times a day for prophylaxis of herpes simplex virus infection until day 100 after SCT. Nineteen CMV-seropositive patients received a transplant from a seropositive donor, and four patients received a transplant from a seronegative donor; another ten CMV-seronegative patients received a transplant from a seropositive donor. Thus, 33 of the 51 patients analyzed were at risk to develop CMV infection and disease. All CMV-seropositive patients and/or patients receiving a transplant from a CMV-seropositive donor received blood products which had not been screened for CMV, whereas all CMV-seronegative patients receiving a transplant from a CMV-seronegative donor received CMV-seronegative blood products. Informed consent was obtained from patients, according to the declaration of Helsinki, and the study was approved by an institutional review board.

Study design. All 51 patients were monitored weekly for signs of CMV reactivation or infection by Murex HCS, PCR with whole blood, and conventional as well as rapid virus culture assays beginning at the time of neutrophil recovery (>500/μl) until day 100 posttransplantation. CMV-seronegative patients receiving a transplant from a CMV-seronegative donor were included as negative controls.

Therapy for CMV infection and CMV disease. All patients were included in a clinical trial used to evaluate PCR-based preemptive therapy with ganciclovir. Murex HCS was not considered for clinical decision making. According to the study protocol, PCR-based preemptive therapy with ganciclovir (two doses of 5

TABLE 1. Clinical characteristics of study patients at transplantation[a]

Characteristic	No. of patients
Sex	
Male	29
Female	22
Underlying disease	
Chronic myeloid leukemia	26
Acute leukemia	15
Myelodysplastic syndrome	8
Inborn errors of immunodeficiency	2
CMV serology	
Donor$^+$/recipient$^+$	19
Donor$^+$/recipient$^-$	4
Donor$^-$/recipient$^+$	10
Donor$^-$/recipient$^-$	18

[a] The mean age of the patients was 33.1 years (range, 2 to 57).

mg/kg of body weight/day) was initiated within 24 to 48 h from the time of the second consecutive positive PCR result as previously described (3). Patients with documented CMV disease received only combination therapy with ganciclovir and CMV-hyperimmunoglobulin. To reduce the duration of antiviral therapy, ganciclovir was stopped after 14 days if clearance of the virus could be documented by PCR. Thus, maintenance therapy with ganciclovir at 5 mg/kg/day five times weekly was administered only when PCR remained positive after a 14-day course of antiviral therapy.

CMV disease. CMV disease was diagnosed according to the criteria defined by the Fourth International Cytomegalovirus Workshop in Paris 1993 (12), as follows. CMV pneumonia was indicated by dyspnea, interstitial infiltrates on the chest radiograph, and positive CMV culture of bronchial washings. CMV enteritis was indicated by gastrointestinal symptoms and the demonstration of CMV by histology or immunohistology. CMV hepatitis was indicated by abnormal liver function, typical histological changes, and the detection of CMV in the liver biopsy by culture or DNA hybridization.

PCR. DNA extraction of 5 ml of EDTA-anticoagulated blood was performed as previously reported (5). A 147-bp DNA fragment located between positions 1767 and 1913 of the fourth exon of the immediate-early gene of the human CMV strain AD169 was amplified by PCR with specific primers. In all assays 0.1, 1, 10, and 100 fg of cloned HCMV-DNA fragment pCM5018 were amplified. The intensity of the signals of the titration curve was compared with the signal from each clinical sample and a semiquantitative assessment was performed as recently described by our group (10). The median time for a complete PCR test was 12 h.

Virus culture. Human foreskin fibroblasts grown in glass tubes were inoculated for 45 min at 37°C with 0.2 ml of a leukocyte suspension (1×10^6 to 2×10^6 cells) prepared by dextran sedimentation. After removal of the clinical material, human foreskin fibroblasts were maintained in culture in minimal essential medium at 37°C. Growth of CMV was identified by production of its characteristic cytopathic effects on human foreskin fibroblasts. In a separate identically inoculated culture, CMV-specific antigens were detected 48 h after inoculation by an indirect peroxidase technique. Cultures were fixed with cold methanol for 10 min. Monoclonal antibody E13 (dilution, 1:750; Biosoft, Paris, France), which is directed against CMV immediate-early antigen, was added for 60 min at 37°C. Subsequent incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody (dilution, 1:750; Dako, Hamburg, Germany) and with the chromogen aminoethylcarbazol (Sigma Chemical Co., St. Louis, Mo.) yielded brown nuclear staining of infected cells. Cultures were read with a Leitz Fluovert microscope at a 63-fold magnification.

Murex HCS. The assay was provided by Murex Diagnostica GmbH (Burgwedel, Germany) and was performed within 8 h according to the manufacturer's instructions. Briefly, 3.5 ml of EDTA-anticoagulated blood was lysed with the manufacturer's lysis solution. The leukocytes were transferred to hybridization tubes, pelleted, and either stored at −20°C for up to 2 months or immediately processed. The denaturing agents were added to the samples for 25 min at 70°C. The samples were transferred to fresh hybridization tubes and incubated for an additional 25 min at 70°C. Thereafter, the CMV-specific RNA probe was added, and the tubes were incubated for 2 h at 70°C. The RNA-DNA hybrids were transferred to specific-antibody-coated capture tubes that were placed on a rotary shaker for 60 min at 1,100 rpm. An alkaline phosphatase-conjugated antibody specific for RNA-DNA hybrids was added, and the tubes were incubated for 30 min at room temperature. The tubes were then washed and the substrate was added; after a further incubation step, the tubes were read with a luminometer. CMV-DNA was quantitated by comparing the relative light units (RLU) of the samples to the RLU of a calibration standard curve, and the results were expressed as genomes per milliliter. A ratio of sample RLU to the positive cutoff value of >1.0 was considered positive for CMV DNA according to the manufacturer's instructions.

TABLE 2. Time to positive PCR, HCS, blood culture, or clinical symptoms in 18 PCR-positive patients[a]

Patient	No. of days posttransplantation to positive result as assessed by:			
	PCR	HCS	Blood culture	CMV disease
1	25	32	49	38 (HP)
2	49	49		60 (IP)
3	34	41	50	46 (IP)
4	19	24	31	24 (IP)
5	14	14		
6	38	42	51	
7	15			
8	68	68		
9	43			
10	37		54	
11	56			
12	36	85	100	
13	50			
14	55			
15	64	64		
16	54	88		
17	35	43		
18	26			
Median (range)	37.5 (14–68)	43 (14–88)	50.5 (31–100)	

[a] Abbreviations: HP, hepatitis; IP, interstitial pneumonia.

Statistical evaluation. Primary study endpoints were the time from SCT to a positive PCR, HCS, and culture test result. Differences in sensitivity were analyzed by log rank analysis for censored data. Comparison of the viral load in patients with symptomatic and asymptomatic CMV infection and with culture-positive and culture-negative blood samples as well as with semiquantitatively assessed PCR- and HCS-positive blood samples was performed by using the Wilcoxon rank test (GraphPad Prism, version 2; GraphPad Software, Inc., San Diego, Calif.).

RESULTS

Diagnosis of CMV infection. A total of 417 blood samples from 51 patients who underwent an allogeneic SCT (mean, 8 samples per patient; range, 3 to 18) were analyzed. CMV viremia was diagnosed in 23 patients by PCR (109 of 417 samples), in 12 patients by HCS (21 of 417 samples), and in 6 patients by the culture method (6 of 417 samples). When the times to the first positive test result were compared in patients who received preemptive antiviral therapy as mandated in the study, PCR proved to be more sensitive than HCS ($P < 0.0001$) and HCS was more sensitive than the culture method ($P < 0.0001$). CMV infection was diagnosed by PCR a median of 37.5 days (mean, 39.9 days; standard error [SE], 3.83 days; range, 14 to 68 days), by HCS a median of 43 days (mean, 50 days; SE, 7.2 days; range, 14 to 88 days), and by blood culture a median of 50.5 days (mean, 55.8 days; SE, 9.4 days; range, 31 to 100 days) posttransplantation (Table 2). None of the 18 CMV-seronegative patients receiving grafts from a seronegative donor had a positive HCS result, and only one had a single slightly positive PCR assay result.

Results were 95.9% concordant between blood cultures and HCS, 75.3% concordant between blood cultures and PCR, and 78.9% concordant between PCR and HCS. All PCR-negative samples were HCS and culture negative. Eighty-eight samples were PCR positive but HCS negative. Of these, 18 discordant samples were taken prior to and 64 after initiation of antiviral

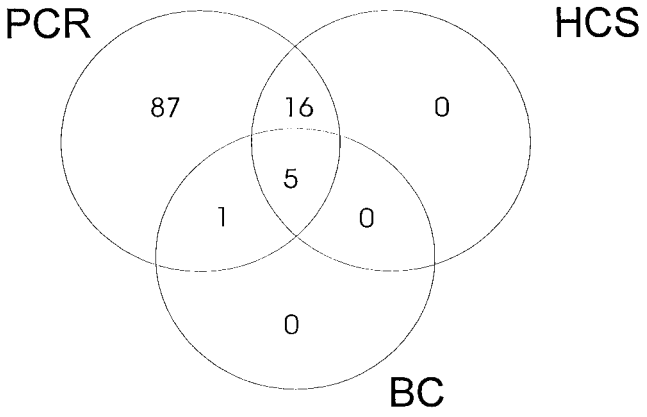

FIG. 1. Venn diagram showing the correlation of test results in 109 blood samples found to be positive in each of the three assays analyzed: PCR (PCR from whole blood), HCS, and blood culture (BC).

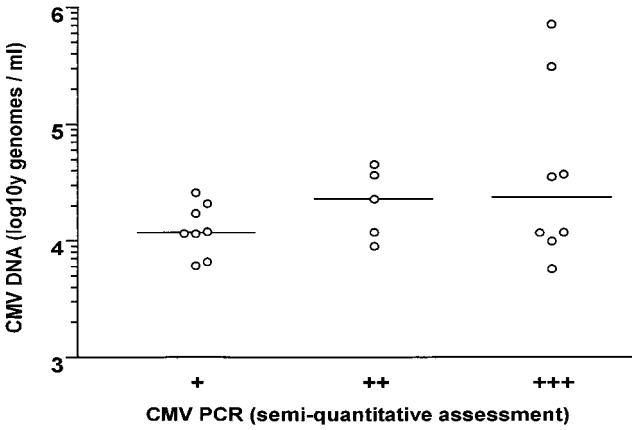

FIG. 2. Evaluation of CMV DNA quantified by HCS (genomes per milliliter) versus semiquantitatively assessed PCR results for 21 HCS-positive blood samples. The median DNA values were 11,770 (PCR^{+}), 22,860 (PCR^{++}), and 23,680 (PCR^{+++}) genomes/ml, respectively (for PCR^{+} versus PCR^{+++}, $P = 0.07$). Horizontal lines represent the median values.

therapy. Because five HCS-negative patients were PCR positive only once, these patients did not receive antiviral therapy. One HCS-negative blood sample proved to be culture positive (Fig. 1). This sample was drawn 10 days after initiation of antiviral therapy and in the absence of clinical signs of CMV disease. Moreover, due to toxicity related to ganciclovir, the leukocyte count had dropped below 4,000/μl in this patient. Additionally, sensitivity was not found to be affected by the leukocyte counts when the viral loads in PCR-positive blood samples between those above and those below 4,000 leukocytes/μl were compared ($P = 0.1$).

When the viral DNA loads quantified by HCS were compared with those found by semiquantitative assessment by PCR (scored as +, ++, and +++) in 21 blood samples positive in both assays, a lower viral load as assessed by HCS was found in samples with a weakly positive PCR signal (PCR^{+}: median, 11,770; mean, 13,980; SE, 2,438; range, 6,097 to 25,900 genomes/ml) than in samples with a moderately positive signal (PCR^{++}: median, 22,860; mean, 25,110; SE, 6,997; range, 9,000 to 45,320 genomes/ml) and those with a strongly positive PCR signal (PCR^{+++}: median, 23,680; mean, 254,100; SE, 89,850; range, 5,772 to 717,500 genomes/ml). However, due to the low number of HCS-positive samples, this result did not reach statistical significance (PCR^{+} versus PCR^{++}, $P = 0.09$; PCR^{+} versus PCR^{+++}, $P = 0.07$) (Fig. 2). Moreover, CMV DNA levels as assessed by HCS were higher in culture-positive than in culture-negative blood samples (culture positive: median, 35,510; mean, 166,400; SE, 137,900; range, 11,530 to 717,500 genomes/ml; culture negative: median, 11,840; mean, 35,120; SE, 18,650; range, 5,772 to 311,700 genomes/ml) ($P = 0.06$).

Clinical course and monitoring of antiviral therapy. Eighteen patients received antiviral therapy until day 100 per study-mandated rules. Four of these 18 patients developed proven CMV disease at the time of the second consecutive positive PCR result (3 had CMV interstitial pneumonia and 1 had CMV hepatitis). Two of these patients had received a transplant from an unrelated donor, and two had received a partially T-cell-depleted graft. All four patients recovered from CMV disease under combined therapy with ganciclovir and CMV hyperimmunoglobulin.

CMV infection was diagnosed by HCS in 12 of 18 patients receiving antiviral therapy based on PCR-positive findings in two consecutive blood samples. All four patients who developed CMV disease were correctly identified by HCS a median of 5.5 days prior to onset of CMV-related symptoms (range, 0 to 11 days), whereas only three were identified by analyzing culture from whole blood (Table 2). The median peak titer of CMV DNA, as measured by the HCS assay, was greater in the four patients with CMV disease (median, 28,190; mean, 197,800; SE 173,300; range, 17,270 to 717,500 genomes/ml) than in patients with asymptomatic CMV infection (median, 11,570; mean, 14,670; SE, 3,595; range, 4,576 to 45,320 genomes/ml) ($P = 0.06$) (Fig. 3).

When CMV disease was used as the standard of comparison, the positive predictive value for a positive HCS assay result was 33.3% and for two consecutive positive PCR results it was 22.2%. The negative predictive value was 100% for both assays.

PCR positivity persisted for a median of 4 weeks (range, 2 to 8 weeks) after initiation of antiviral therapy, whereas HCS was found to be negative in one patient after 3 weeks, in three patients after 2 weeks, and in 8 patients after 1 week of antiviral therapy. All patients with asymptomatic CMV infection were HCS negative 1 week after initiation of antiviral therapy.

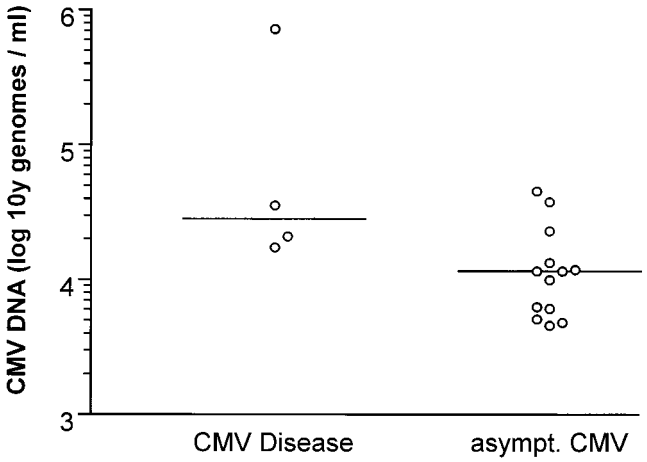

FIG. 3. Relationship between CMV DNA and the severity of CMV infection. Horizontal lines represent the median values.

Thus, the efficacy of antiviral therapy could be monitored by using HCS and PCR.

DISCUSSION

To determine whether CMV HCS is sensitive enough to initiate and monitor antiviral therapy after allogeneic SCT, 51 patients were prospectively screened by HCS, a homemade PCR assay, and a blood culture technique. As expected, PCR was more sensitive than HCS ($P < 0.0001$) and HCS was more sensitive than viral blood culture ($P < 0.0001$). PCR and HCS findings and HCS and blood culture findings were found to be concordant in 78.9 and 95.9% of the samples, respectively. All patients with CMV disease were correctly identified by HCS and PCR. The viral burden was found to be higher in patients with CMV disease and those with a positive blood culture assay ($P = 0.06$). HCS results became negative after an average of 8 days of antiviral therapy and remained positive for up to 3 weeks in patients with CMV-related symptoms, indicating that HCS might be appropriate for monitoring the effectivity of antiviral drugs.

HCS has been shown to allow the rapid, quantitative, and objective measurement of the CMV load in HIV-infected patients and in recipients of an organ allograft (11, 13, 14, 18). Moreover, a reasonable quantitative correlation of HCS and pp65 antigenemia has been demonstrated in patients with a high viral burden, whereas patients with low-level antigenemia (<20 positive/10^5 cells) were less likely to be HCS positive, indicating that HCS is less sensitive than the pp65 antigen assay (14, 18). Our homemade PCR assay performed with DNA extracted from whole blood showed a sensitivity comparable to that of PCR performed on DNA extracted from plasma or pp65 antigenemia following SCT (10). Thus, the higher sensitivity of the PCR than of HCS was expected.

Preemptive antiviral therapy based on PCR has been shown to significantly reduce CMV-related morbidity and mortality (3). To further reduce the duration of potentially toxic antiviral therapy, PCR-based preemptive ganciclovir is discontinued at our institution once patients have cleared the virus from the blood after 2 to 4 weeks of therapy (3). A much lower number of samples were found to be positive by HCS than by PCR prior to and also after initiation of antiviral therapy. Since all patients received preemptive antiviral therapy after the second consecutive positive PCR assay irrespective of their HCS status, the prevalence of a positive HCS result following allogeneic SCT may have been underestimated. Thus, whether HCS is suitable to initiate and monitor antiviral therapy in recipients of an allogeneic SCT remains debatable. However, because of the very high mortality of CMV disease in recipients of an allogeneic SCT (15), the lower sensitivity limit of HCS may be critical in allowing a diagnosis of CMV infection early enough in this patient cohort. Four patients developed CMV disease at the time of the second consecutive positive PCR result. Three of these four patients were found to be HCS positive prior to and one patient was positive at the onset of clinical symptoms, whereas only three of four patients were correctly identified by the blood culture assay after the onset of clinical symptoms. Thus, HCS was more sensitive in identifying patients with CMV disease than the blood culture assay.

As methods for CMV detection become more sensitive, quantitation of CMV is becoming more important in assessing the predictive value of a positive test result. In solid organ transplant recipients and in patients with AIDS, CMV-related symptoms are associated with a high viral burden in the blood, whereas in recipients of a bone marrow transplant, even fatal disease does not seem to be associated with higher levels of virus in blood leukocytes (11, 13, 17). More-sensitive quantitative blood assays in recipients of an allogeneic SCT may help to define a threshold and thus reduce the incidence of overtreatment (8, 20). In this study, we have demonstrated that quantitation of the CMV load in the blood can be easily achieved by HCS. Moreover, a high viral burden correlated with CMV-related symptoms. In contrast, because quantitative competitive PCR protocols are still time-consuming and labor-intensive and are not a part of the daily routine in most facilities, only limited data for these assays are currently available.

HCS allows storage of samples for several days without a loss of sensitivity (14), in contrast to the pp65 antigen assay, which requires immediate sample processing (2). Moreover, quantitative PCR and pp65 antigenemia analyses are still poorly standardized. Recently, a quantitative plasma PCR plate assay adopting enzyme-linked immunosorbent assay technology to measure the amount of CMV DNA was developed, and a good correlation with a quantitative competitive PCR assay was demonstrated for this method (7). Further prospective comparisons of different methods are needed to achieve a better standardization of CMV diagnosis and treatment monitoring.

In conclusion, HCS was more sensitive than the blood culture technique but less sensitive than the PCR assay. HCS can be used to monitor the efficacy of antiviral therapy by quantitation of CMV DNA in the blood. The short interval between a positive test result and the onset of CMV-related symptoms indicates that the sensitivity of the assay is still insufficient to allow initiation of antiviral therapy in recipients of an allogeneic SCT early enough to prevent CMV disease. A second-generation HCS with improved sensitivity has been developed and is being assessed for patients following allogeneic SCT in an ongoing study.

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft (DFG) grant SFB 510 B3.

CMV HCS DNA kits (version 1) were kindly provided by Murex.

REFERENCES

1. **Boeckh, M., T. A. Gooley, D. Myerson, T. Cunningham, G. Schoch, and R. A. Bowden.** 1996. Cytomegalovirus pp65 antigenemia guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood **88:**4063–4071.
2. **Boeckh, M., M. Woogerd, T. Steven-Ayers, C. G. Ray, and R. A. Bowden.** 1994. Factors influencing detection of quantitative cytomegalovirus antigenemia. J. Clin. Microbiol. **32:**832–834.
3. **Einsele, H., G. Ehninger, H. Hebart, et al.** 1995. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood **86:**2815–2820.
4. **Einsele, H., G. Ehninger, M. Steidle, A. Vallbracht, M. Muller, H. Schmidt, J. G. Saal, H. D. Waller, and C. A. Muller.** 1991. Polymerase chain reaction to evaluate antiviral therapy for cytomegalovirus disease. Lancet **338:**1170–1172.
5. **Einsele, H., M. Steidle, A. Vallbracht, J. G. Saal, G. Ehninger, and C. A. Müller.** 1991. Early occurrence of HCMV infection after BMT as demonstrated by the PCR technique. Blood **77:**1104–1110.
6. **Emanuel, D., I. Cunningham, K. Jules-Elysee, et al.** 1988. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. Ann. Intern. Med. **109:**777–782.
7. **Gallez-Hawkins, G. M., B. R. Tegtmeier, A. Veer, J. C. Niland, S. J. Forman, and J. A. Zaia.** 1997. Evaluation of a quantitative plasma PCR plate assay for detecting cytomegalovirus infection in marrow transplant recipients. J. Clin. Microbiol. **35:**788–790.
8. **Gerna, G., M. Furioni, F. Baldanti, E. Percivalle, P. Comoli, and F. Locatelli.** 1995. Quantitation of human cytomegalovirus DNA in bone marrow transplant recipients. Br. J. Haematol. **91:**674–683.
9. **Grundy, J. E., A. Ehrnst, H. Einsele, V. Emery, H. Hebart, G. Prentice, and P. Ljungman.** 1996. A three-center European external quality control study of PCR for detection of cytomegalovirus DNA in blood. J. Clin. Microbiol. **34:**1166–1170.

10. **Hebart, H., C. Müller, J. Löffler, G. Jahn, and H. Einsele.** 1996. Monitoring of CMV infection: a comparison of PCR from whole blood, plasma-PCR, pp65-antigenemia and virus culture in patients after bone marrow transplantation. Bone Marrow Transplant. **17:**861–868.
11. **Imbert-Marcille, B. M., D. Cantarovich, V. Ferreaubineau, B. Richet, J. P. Soulillou, and S. Billaudel.** 1997. Usefulness of DNA viral load quantification for cytomegalovirus disease monitoring in renal and pancreas/renal transplant recipients. Transplantation **63:**1476–1481.
12. **Ljungman, P., and P. Griffiths.** 1993. Definitions of cytomegalovirus infection and disease, p. 233–237. *In* S. Michelson and S. A. Plotkin (ed.), Multidisciplinary approach to understanding cytomegalovirus disease. Elsevier Science Publishers B. V., Paris, France.
13. **Macartney, M., E. J. Gane, B. Portmann, and R. Williams.** 1997. Comparison of a new quantitative cytomegalovirus DNA assay with other detection methods. Transplantation **63:**1803–1807.
14. **Mazzulli, T., S. Wood, R. Chua, and S. Walmsley.** 1996. Evaluation of the Digene capture system for detection and quantitation of human cytomegalovirus viremia in human immunodeficiency virus-infected patients. J. Clin. Microbiol. **34:**2959–2962.
15. **Meyers, E. D., N. Flournoy, and E. Thomas.** 1986. Risk factors for cytomegalovirus infection after human bone marrow transplantation. J. Infect. Dis. **153:**478–488.
16. **Reed, E. C., R. A. Bowden, P. S. Dandliker, K. E. Lilleby, and J. D. Meyers.** 1988. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplantation. Ann. Intern. Med. **109:**783–788.
17. **Saltzman, R. L., M. R. Quirk, and M. C. Jordan.** 1992. High levels of circulating cytomegalovirus DNA reflect visceral organ disease in viremic immunosuppressed patients other than marrow recipients. J. Clin. Invest. **90:**1832–1838.
18. **Veal, N., C. Payan, D. Fray, L. Sarol, O. Blanchet, S. Kouyoumdjian, and F. Lunel.** 1996. Novel DNA assay for cytomegalovirus detection: comparison with conventional culture and pp65 antigenemia assay. J. Clin. Microbiol. **34:**3097–3100.
19. **Wolf, D. G., and S. A. Spector.** 1993. Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. Transplantation **56:**330–334.
20. **Zipeto, D., F. Baldanti, D. Zella, M. Furione, A. Cavicchini, G. Milanesi, and G. Gerna.** 1993. Quantification of human cytomegalovirus DNA in blood polymorphonuclear leukocytes of immunocompromised patients by the polymerase chain reaction. J. Virol. Methods **44:**45–55.