Risk factors for the development of cytomegalovirus disease after allogeneic stem cell transplantation

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Background and Objectives. Cytomegalovirus (CMV) disease remains an important complication of allogeneic stem cell transplantation (SCT). We studied viral load kinetics and correlated the viral load and other transplant factors with the development of CMV disease.

Design and Methods. We studied 162 consecutive patients who were CMV seropositive or had CMV seropositive donors. Quantification of CMV DNA was performed by real-time polymerase chain reaction.

Results. CMV DNA detected was detected in 105 of the 162 patients. The mean peak viral loads were similar at first and subsequent reactivations. The serologic status of the donors and recipients prior to SCT significantly influenced the viral load. The cumulative incidence of CMV disease was 1.8% at 100 days and 6.3% at 365 days after SCT. The peak viral load were higher in patients who developed CMV disease than in patients without CMV disease (log_{10} 3.5; SE ± 0.26/200,000 cells vs. log_{10} 2.7; SE ± 0.09/200,000 cells; p=0.02). However, in multivariate analysis, only acute graft-versus-host disease (GVHD) grade II-IV and a graft from a CMV-negative donor to a CMV-positive patient were significant risk factors for CMV disease. In patients who required more than one course of pre-emptive therapy, acute GVHD and the rate of decrease in viral load during first pre-emptive therapy were significant risk factors for subsequent development of CMV disease.

Interpretation and Conclusions. A decrease in viral load during pre-emptive therapy is an important factor for later development of CMV disease.

Key words: CMV, monitoring, PCR, stem cell transplantation.

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Viremia is a well-recognized risk factor for cytomegalovirus (CMV) disease after allogeneic stem cell transplantation (SCT) and the strategy of pre-emptive antiviral therapy has been shown to reduce the risk of CMV disease.[1,2] Different diagnostic techniques have been used to detected CMV, including shell vial culture, pp65 antigenemia, qualitative and quantitative polymerase chain reaction (PCR) analyses for CMV DNA, and detection of CMV mRNA.[1,3-6] Viral load kinetics has been reported to be predictive for the development of CMV disease with the initial viral load and the initial rate of increase in viral load being independent risk factors.[7] During recent years new techniques that have been introduced in stem cell transplantation, such as grafts of peripheral blood stem cells and reduced intensity conditioning, have partly changed the timing of immune reconstitution after SCT and thereby might have changed the predictive value of viral load kinetics. The aims of this study were to analyze the effects of different pre-transplant related factors on viral load and the effects of the viral load and viral load kinetics on the risk of CMV disease in a series of consecutive allogeneic SCT recipients.

Design and Methods

Patients

One hundred and sixty-two consecutive patients transplanted at Huddinge University Hospital between January 1, 2000, and December 31, 2003 were included in the study. The patients were CMV-seropositive or had received stem cells from CMV seropositive donors. The study was approved by the ethical committee at Huddinge University Hospital. The patients' characteristics are shown in Table 1.

Stem cell transplant procedure

The conditioning regimens have been previously described.[8-10] Myeloablative regimens were given with either 12 Gy of fractionated total body irradiation combined with cyclophosphamide 60 mg/kg for two consecutive days or busulphan 4 mg/kg on 4 consecutive days combined with cyclophosphamide 60 mg/kg for 2 consecutive days. All patients with an unrelated or mismatched related donor were treated with antithymocyte globulin (ATG; 2-5 mg/kg per day) for 2 to 5 days

before transplantation. The standard reduced intensity regimen was fludarabine (30 mg/m^2/day) for 6 days, combined with 4 mg/kg/day of busulphan for 2 days and ATG (2 mg/kg/day) for 4 days but other regimens were also used.[10]

Graft-versus-host disease prophylaxis and therapy (GVHD)

The vast majority of the patients received cyclosporine A combined with four doses of methotrexate.[11] Cyclosporine A was discontinued after 3 months (sibling donors) or 6 months (unrelated or mismatched donors), if possible. Both acute and chronic GVHD were diagnosed based on clinical symptoms and/or biopsy samples from skin, liver, gastro-intestinal tract, or oral mucosa. Patients were treated for grade I acute GVHD with prednisolone starting at a dose of 2 mg/kg/days, which was then tapered down after the initial response. In more severe cases, methylprednisolone, ATG, methotrexate, and/or psoralen and ultra-violet A (PUVA) or extracorporeal PUVA was given. Several strategies were used to treat extensive chronic GVHD, including prednisolone, cyclosporine A, and prednisolone on alternate days, mycophenolate mofetil, PUVA or extracorporeal PUVA, thalidomide, or total lymph node irradiation.

Supportive care and treatment

Prophylaxis against infections included the oral ciprofloxacin, oral amphotericin B, and acyclovir during the neutropenic phase.[12] Most patients were treated in hospital using reversed isolation. However, some patients living within a 2-hour drive from the hospital were treated at home[13] and some patients receiving reduced conditioning regimens were treated as outpatients. Cotrimoxazole was administered as prophylaxis against *P. jirovecci* during conditioning until 2 days before transplantation, restarted after engraftment and continued for at least 6 months. Patients with chronic GVHD received prolonged prophylaxis with cotrimoxazole.

Monitoring for CMV

The patients were monitored at least once weekly from engraftment until day 100 after SCT. Following this period, patients who had experienced CMV reactivation or had severe GVHD continued to be monitored weekly while other patients were monitored at each visit to the transplant center (usually every 2 weeks) until 6 months after SCT.

Quantitative PCR for CMV DNA

The quantitative real-time PCR used for quantification of CMV DNA from peripheral blood lymphocytes has been previously described.[14] CMV disease was defined according to Ljungman *et al.*[15]

Pre-emptive antiviral therapy

Preemptive therapy with either intravenous ganciclovir or foscarnet was given as described by Reusser *et al.*[16] Although different triggers for initiating therapy were used during this study. During a randomized study between PCR and detection of late mRNA (NASBA), therapy was initiated either after detection of at least 10 copies of CMV DNA/200,000 cells in two consecutive samples or detection of mRNA in one sample.[17] After May 1, 2002 a viral load of 100 genome copies/200,000 cells in one sample was used as the basis for initiating pre-emptive antiviral therapy. Treatment was given for 2 weeks at full dose (usually ganciclovir 5 mg/kg×2) and if CMV was above 10 or 100 genome copies, respectively, therapy was given as maintenance for another 2 weeks (6 mg kg×1) as previously reported.[16,18] Repeated courses were given as necessary based on a new episode of CMV DNAemia/RNAemia. For the purpose of this study, a second reactivation had to be preceded by negative monitoring results for at least 8 days in the absence of antiviral therapy.

Table 1. Patients' characteristics.

Characteristic	
Median age (range)	38.4 (0.5-64.5)
Underlying disease	
Acute myeloid leukemia	52
Acute lymphocytic leukemia	24
Chronic myeloid leukemia	21
Myelodysplastic syndrome	17
Lymphoma including chronic lymphocytic leukemia	15
Myeloma	9
Severe aplastic anemia	8
Solid tumors	6
Others	10
Donor type	
Matched sibling	64
Unrelated	93
Mismatched family	5
Stem cell source	
Bone marrow	50
Peripheral blood stem cells	109
Cord blood	3
Conditioning	
Myeloablative	97
Reduced	65
CMV serological status	
Donor +/recipient +	82
Donor –/recipient +	59
Donor +/recipient –	21

Statistics

All viral load results were transformed to $\log_{10}$ values. The kinetics of viral load changes during the first reactivation was calculated as the $\log_{10}$ of the first positive sample – a nominal $\log_{10}$ value of 0.699 was ascribed to the last previous negative sample divided by the number of days between the two samples.[7] The kinetics of viral load changes during the first course of antiviral therapy was calculated as the $\log_{10}$

viral load at initiation of antiviral therapy – the log_{10} viral load of the last sample before antiviral therapy was discontinued divided by the number of days of antiviral therapy. t-tests were used for comparing viral loads. Multivariate logistic regression analysis was used to evaluate the effects of different factors on the risk of CMV disease. Factors with a *p* value of ≤0.1 were included in the multivariate analyses. The cumulative incidence for CMV disease was calculated using death and relapse as competing risks.

Results

CMV replication

CMV DNA was detected by PCR at least once in 105 of the 162 (65.0%) patients. The mean time between SCT and the first positive PCR was 26 days. There was no impact on the time to first positive PCR according to the source of stem cells, reduced intensity or standard conditioning, donor type, or the donor/recipient serostatus combinations. Reactivation occurred once in 69 patients, twice in 31 and three times in five patients. The mean peak viral loads were similar in the first CMV reactivation (mean log_{10} DNA copies 2.7±0.08) and subsequent reactivations (2.7±0.12 for the second reactivatios and 2.8±0.07 for the third reactivation). There was a strong correlation between the viral load in the first positive sample and the peak viral load (r=0.89; $p<0.0001$). There was no difference in overall survival between patients who did or a did not have a CMV reactivation (65% vs. 63% at 12 months after SCT; *p*=ns).

The monitoring strategy used influenced the peak viral loads. Patients monitored by mRNA had the highest peak viral load (mean log_{10} DNA copies 3.4±0.24) compared to patients requiring two consecutive positive samples before initiation of antiviral therapy (mean log_{10} DNA copies 3.0±0.16) and patients whose therapy was started after one positive sample mean log_{10} DNA copies 2.5±0.09).

Pre-emptive antiviral therapy

Ninety-two patients (56.7%), who accounted for 87.6% of patients with CMV DNA detected at any time, received pre-emptive antiviral therapy. Thirteen patients with CMV reactivation did not receive antiviral therapy because of low viral loads or only one positive sample. None of these patients developed CMV disease.

Fifty-six patients received one course of antiviral therapy, 31 received two, and five received three courses of pre-emptive antiviral therapy. The mean number of weeks of antiviral therapy was four for patients with one reactivation, six for patients with two reactivations, and 12 for patients with three reactivations. Whether the patient was given only induction or induction and maintenance therapy had no influence on the risk of a second reactivation (*data not shown*). Ganciclovir was used in 89 of the first courses while foscarnet was used in three courses. Ganciclovir was used in 19 second line courses, foscarnet in 11, valganciclovir in four, and cidofovir in two second line courses while foscarnet was given in two third line courses and valganciclovir in three. In addition, valacyclovir was used in 14 patients (in 11 after two courses and in three after three courses of pre-emptive therapy) with the aim of avoiding additional pre-emptive therapy. This strategy was successful in 11 of 14 patients while three patients required intravenous therapy. None of the patients receiving valacyclovir as secondary prophylaxis developed CMV disease.

Table 2. Viral loads and different transplant-related factors.

Factor	*Log_{10} DNA copies; mean ± SE)*	*p-value*
Donor/recipient serology		
Donor+/Recipient+ (1)	3.3 (0.39)	(1) v (2) *p*=NS
Donor+/Recipient- (2)	3.0 (0.16)	(2) v (3)*p*=<0.05
Donor-/Recipient- (3)	2.6 (0.10)	(1) v (3) *p*=<0.05
Donor type		
Unrelated/mis-matched	2.7 (0.09)	NS
Sibling donor	2.9 (0.17)	
Conditioning		
Myeloablative	3.0 (0.13)	NS
Reduced intensity	2.7 (0.14)	
Stem cell source		
Bone marrow	2.8 (0.20)	
Peripheral blood stem cells	2.8 (0.1)	NS
Cord blood	2.8 (0.79)	

Peak viral load and transplant factors

The patients' peak viral loads and different transplant factors are shown in Table 2. Donor/patient serologic status before SCT significantly influenced the viral load while donor type, conditioning, and stem cell source did not.

CMV disease

Ten of 162 patients developed CMV disease; three patients had interstitial pneumonia, four patients had gastrointestinal disease, and three patients developed CMV retinitis. The median time to CMV disease was 104 days (range 39-200). Five patients developed *late* CMV disease, defined as disease diagnosed after day 100. The cumulative incidence of CMV disease was 1.8% at day 100 and 6.3% at day 365. Three of the 10 patients died from the CMV disease. The mortality from CMV disease in the entire group was 2.0%. Only one patient developed CMV disease without having previously received anti-CMV therapy. Eight of 10 patients with CMV disease had received at least one full course of pre-emptive antiviral therapy before development of CMV disease while one patient had been receiving antiviral therapy for 3 days when CMV disease was diagnosed.

Risk factors for CMV disease

Patients with acute GVHD grades II-IV were more likely to develop CMV disease (7 of 39; 17.9%) than were patients with acute GVHD grades 0-I (3 of 123; 2.4%; $p<0.01$). No patient developed CMV disease before the diagnosis of acute GVHD. Patients who were CMV seropositive but had negative donors were slightly more likely to develop CMV disease (6 of 59; 10.1%) than were seropositive patients with seropositive donors (3 of 82; 3.6%) or seronegative patients with seropositive donors (1 of 21; 4.7%; $p=0.1$). Monitoring strategy (PCR or mRNA) had no effect on the risk of CMV disease (*data not shown*). There was also no difference in the rate of CMV disease between patients who received myeloablative (7 of 97; 8.9%) or reduced intensity (3 of 65; 4.6%) conditioning, between patients who did or did not receive ATG in their conditioning (*data not shown*), or between patients receiving grafts from mismatched or unrelated donors (5 of 98; 5.1%) or sibling donors (5 of 64; 7.8%).

Patients who developed CMV disease had higher peak viral loads in the first episode of CMV-DNAemia ($\log_{10}$ 3.5; SE±0.26; $p=0.02$) than did patients without CMV disease ($\log_{10}$ 2.7; SE±0.09). However, due to initiation of antiviral therapy before the diagnosis of disease, the viral load at the time of diagnosis of CMV disease was lower ($\log_{10}$ 2.9; SE±0.25). The initial viral load, the viral load at initiation of pre-emptive antiviral therapy, and the rate of increase in viral load during the first reactivation were not different in patients who did or did not develop CMV disease.

In multivariate analysis, acute GVHD (OR 9.7; 1.9-49.8; $p=0.006$) and donor-negative/recipient-positive serological status (OR 5.4; 1.01–29.6; $p=0.049$) were the only risk factors for CMV disease. Viral loads were included in different ways in the multivariate models (initial viral load, peak viral load, viral load at initiation of therapy, the rate of increase in viral load) and none of the measurements had a significant impact on the risk of CMV disease when corrected for GVHD and donor/recipient status. There was also no effect of viral load on the risk of CMV disease if acute GVHD was excluded from the model (*data not shown*).

Viral load kinetics during antiviral therapy

Peak viral load had an influence on the duration of antiviral therapy. Patients with higher viral loads received a longer course of antiviral therapy during their first pre-emptive therapy ($p=0.02$). The viral load decrease during antiviral therapy was calculated for the first course of pre-emptive therapy. In this analysis the two patients who developed CMV disease before or early during the first course of pre-emptive therapy, were excluded. Patients who developed CMV disease had a smaller decrease in viral load ($\log_{10}$ 0.40±0.13/week than patients who did not ($\log_{10}$ 0.66±0.06/week). In multivariate analysis, acute GVHD grades II-IV (OR 11.2; 1.2-73; $p=0.009$) increased the risk and a larger decrease in viral load during antiviral therapy reduced the risk of CMV disease (OR 0.08; 0.01-0.8; $p=0.03$). Donor/recipient serological status, initial viral load, peak viral load, and monitoring strategy had no significant influence on the risk.

Discussion

Monitoring for CMV in peripheral blood combined with the use of pre-emptive antiviral therapy is a successful strategy to reduce the risk of CMV disease. The usefulness of different monitoring techniques depends on their positive and negative predictive values. A very sensitive monitoring technique, such as qualitative PCR might have a low positive predictive value but a very high negative predictive value and, therefore, antiviral therapy might be given unnecessarily to many patients. Despite this risk of overtreatment, Einsele *et al*. were able to show an improved outcome from using PCR to monitor allogeneic SCT patients.[3] The introduction of viral load measurements with quantitative PCR might allow a better selection of patients more likely to develop CMV disease. Emery *et al*. showed that both the initial viral loads and the change in viral loads were independent risk factors for the development of CMV disease not only in allogeneic SCT patients but also in kidney and liver transplant recipients.[7]

The results of the present study are different from those of Emery *et al*.[7] since we could not find that either the initial viral load or the rate of increase in viral load had a significant effect. We did, however, find in univariate analysis that patients with CMV disease had higher peak viral loads than patients who did not develop CMV disease. However, in a multivariate analysis only acute GVHD grades II-IV and the use of CMV seronegative donors for CMV seropositive recipients had significant influences on the risk while the viral load did not. There are several possible reasons for why our results differ from those of Emery *et al*., but the most likely is that Emery *et al*. did not use pre-emptive therapy in the majority of their patients.[7] Thus, the viral load was allowed to increase unhampered by antiviral therapy and thereby the frequency of CMV disease in the cohort studies was much higher. There are also other differences, such as the fact that the majority of patients investigated by Emery *et al*. were T-cell depleted. Finally, we used PCR based on peripheral blood lymphocytes while they used a whole blood PCR. Nevertheless, we do not think this is the explanation since we recently performed parallel analyses on 200 prospectively collected samples comparing the peripheral blood lymphocytes-based PCR with a whole blood-based PCR, showing that the correlation between the viral loads measured by the two techniques is excellent (*Yun and Ljungman; unpublished results*).

Many patients developed repeated episodes of CMV reactivation. Our regimen of antiviral therapy was short and it has been previously shown that

additional reactivations are common.[16,19,20] The peak viral loads were very similar each time supporting the belief that repeated antiviral therapy courses were similarly efficacious. Most patients who developed CMV disease had, therefore, already been treated pre-emptively at least once and developed CMV disease during a second or later reactivation supporting previous data that CMV-specific immunity is a key factor in the control of CMV[21-25] and that antiviral therapy is only a temporary measure until specific immunity has developed. A very interesting finding was that the quality of the response to antiviral therapy differed between patients who did or did not develop CMV disease. A slow response seemed to predispose to later development of CMV disease and was an independent risk factor when controlled for acute GVHD. This could be of practical importance since it is possible that such patients would benefit from prolonged antiviral therapy. We used different antiviral drugs for pre-emptive therapy and all seemed to have similar efficacy. Thus, the likelihood of a repeated reactivation did not seem to depend on the drug used.[16]

It should be recognized that the overall risk for CMV disease in this study was low and the mortality in CMV disease was only 2%. We were unable to find the high frequency of late CMV disease seen in other studies.[21] Why there are such differences between centers is unknown. The selection of patients undergoing transplants is most likely one reason since using more mismatched donors is a risk for the development of severe acute and chronic GVHD. However, other factors such as use of antiviral therapy and GVHD prophylaxis and therapy might also be of importance.

The risk factors for CMV disease that we did identify are not surprising. The correlation between CMV disease and acute GVHD has been known for a long time.[26] The use of corticosteroids is also a risk factor for CMV disease.[7,27] That a CMV seronegative donor for a CMV seropositive patient is a risk factor for CMV disease has not been shown before.[28] However, it has been shown that this serological combination results in poorer survival and higher transplant-related mortality among patients undergoing unrelated donor SCT.[29,30] Both GVHD and using a seronegative donor most likely represent a lack of immune control of CMV.

Another aim of this study was to look at the more recently introduced transplant techniques and their effects on the risk of CMV disease. We were unable to show any effect of graft type or intensity of conditioning. It has been previously shown that reduced intensity conditioning regimens might delay the development of CMV disease but that the overall risks are similar between patients receiving reduced intensity and ablative conditioning.[31,32] These findings fit with the results of our study in which we did not find a lower risk of CMV disease in patients who underwent reduced intensity conditioning. Hakki *et al*. showed that CMV-specific immune reconstitution was later following bone marrow transplantation than after peripheral stem cell transplantation.[27] However, we found neither an increased viral load nor an increased risk of CMV disease in patients receiving bone marrow rather than stem cell grafts.

Probably the most important finding of our study was that viral load kinetics after initiation of antiviral therapy was predictive of the risk of developing CMV disease. Patients who responded slower had a higher risk. Therefore assessment of the antiviral response might be used for deciding management strategies after the first course of pre-emptive antiviral therapy.

PL: designed the study, clinical management of patients, analyzed the data, wrote the paper; LP-Band JJ: chart review, analyzed data, reviewed the paper; GA: discussion of results, reviewed the paper; ES: contributed to design, discussion of results, reviewed the paper; KL: contributed to design, clinical management of patients, discussion of results, reviewed the paper; LB, JA and JW: clinical management of patients, discussion of results, reviewed the paper; ZY: developed the PCR assay, discussion of results, reviewed the paper; OR: clinical management of patients, responsible for the transplant program, discussion of results, reviewed the paper. The authors reported no potential conflicts of interest.

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References

1. Goodrich JM, Mori M, Gleaves CA, Du Mond C, Cays M, Ebeling DF, et al. Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. N Engl J Med 1991;325:1601-7.
2. Meyers JD, Ljungman P, Fisher LD. Cytomegalovirus excretion as a predictor of cytomegalovirus disease after marrow transplantation: importance of cytomegalovirus viremia. J Infect Dis 1990;162:373-80.
3. Einsele H, Ehninger G, Hebart H, Wittkowski KM, Schuler U, Jahn G, et al. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 1995;86:2815-20.
4. Gerna G, Baldanti F, Middeldorp J, Lilleri D. Use of CMV transcripts for monitoring of CMV infections in transplant recipients. Int J Antimicrob Agents 2000;16:455-60.
5. Yun Z, Lewensohn-Fuchs I, Ljungman P, Vahlne A. Real-time monitoring of cytomegalovirus infections after stem cell transplantation using the TaqMan polymerase chain reaction assays. Transplantation 2000;69:1733-6.
6. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood 1996;88:4063-71.
7. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet 2000;355:2032-6.
8. Ringdén O, Ruutu T, Remberger M, Nikoskelainen J, Volin L, Vindelöv L, et al. A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. Blood 1994; 83:2723-30.
9. Svenberg P, Remberger M, Svennilson J, Mattsson J, Leblanc K, Gustafsson B, et al. Allogenic stem cell transplantation for nonmalignant disorders using matched unrelated donors. Biol Blood

Marrow Transplant 2004;10:877-82.
10. Le Blanc K, Remberger M, Uzunel M, Mattsson J, Barkholt L, Ringden O. A comparison of nonmyeloablative and reduced-intensity conditioning for allogeneic stem-cell transplantation. Transplantation 2004;78:1014-20.
11. Ringdén O, Pihlstedt P, Markling L, Aschan J, Båryd I, Ljungman P, et al. Prevention of graft-versus-host disease with T-cell depletion or cyclosporin and methotrexate. A randomized trial in adult leukemic marrow recipients. Bone Marrow Transplant 1991;7:221-6.
12. Ringdén O, Remberger M, Persson U, Ljungman P, Aldener A, Andström E, et al. Similar incidence of graft-versus-host disease using HLA-A, -B and -DR identical unrelated bone marrow donors as with HLA-identical siblings. Bone Marrow Transplant 1995; 15: 619-25.
13. Svahn BM, Remberger M, Myrback KE, Holmberg K, Eriksson B, Hentschke P, et al. Home care during the pancytopenic phase after allogeneic hematopoietic stem cell transplantation is advantageous compared with hospital care. Blood 2002;100:4317-24.
14. Yun Z, Lewensohn-Fuchs I, Ljungman P, Ringholm L, Jonsson J, Albert J. A real-time TaqMan PCR for routine quantitation of cytomegalovirus DNA in crude leukocyte lysates from stem cell transplant patients. J Virol Methods 2003;110:73-9.
15. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002;34:1094-7.
16. Reusser P, Einsele H, Lee J, Volin L, Rovira M, Engelhard D, et al. Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. Blood 2002;99:1159-64.
17. Hebart H, Ljungman P, Klingebiel T, Loeffler J, Lewensohhn-Fuchs I, Barkholt L, et al. Prospective comparison of PCR-based versus late mRNA-based preemptive antiviral therapy for HCMV infection in patients after allogeneic stem cell transplantation. Blood 2003;102:195a[abstract].
18. Ljungman P, Loré K, Aschan J, Klaesson S, Lewensohn-Fuchs I, Lönnqvist B, et al. Use of a semi-quantitative PCR for cytomegalovirus DNA as a basis for pre-emptive antiviral therapy in allogeneic bone marrow transplant patients. Bone Marrow Transplant 1996;17:583-7.
19. Einsele H, Hebart H, Kauffmann-Schneider C, Sinzger C, Jahn G, Bader P, et al. Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. Bone Marrow Transplant 2000; 25:757-63.
20. Ljungman P, Deliliers GL, Platzbecker U, Matthes-Martin S, Bacigalupo A, Einsele H, et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Blood 2001;97:388-92.
21. Boeckh M, Leisenring W, Riddell SR, Bowden RA, Huang ML, Myerson D, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. Blood 2003;101:407-14.
22. Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Loffler J, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood 2002;99:3916-22.
23. Gratama JW, van Esser JW, Lamers CH, Tournay C, Lowenberg B, Bolhuis RL, et al. Tetramer-based quantification of cytomegalovirus (CMV)-specific CD8(+) T lymphocytes in T-cell-depleted stem cell grafts and after transplantation may identify patients at risk for progressive CMV infection. Blood 2001;98:1358-64.
24. Reusser P, Riddell SR, Meyers JD, Greenberg PD. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. Blood 1991; 78: 1373-80.
25. Ljungman P, Aschan J, Azinge JN, Brandt L, Ehrnst A, Hammarstrom V, et al. Cytomegalovirus viraemia and specific T-helper cell responses as predictors of disease after allogeneic marrow transplantation. Br J Haematol 1993;83:118-24.
26. Miller W, Flynn P, McCullough J, Balfour HH Jr., Goldman A, Haake R, et al. Cytomegalovirus infection after bone marrow transplantation: an association with acute graft-v-host disease. Blood 1986;67:1162-7.
27. Hakki M, Riddell SR, Storek J, Carter RA, Stevens-Ayers T, Sudour P, et al. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. Blood 2003;102:3060-7.
28. Boeckh M, Nichols WG. The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. Blood 2004;103:2003-8.
29. Ringden O, Schaffer M, Le Blanc K, Persson U, Hauzenberger D, Abedi MR, et al. Which donor should be chosen for hematopoietic stem cell transplantation among unrelated HLA-A, -B, and -DRB1 genomically identical volunteers? Biol Blood Marrow Transplant 2004;10:128-34.
30. Ljungman P, Einsele H, Frassoni F, Niederwieser D, Cordonnier C. Donor CMV serological status influences the outcome of CMV seropositive recipients after unrelated donor stem cell transplantation; an EBMT Megafile analysis. Blood 2003;102:4255-60.
31. Junghanss C, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG, et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. Blood 2002;99:1978-85.
32. Schetelig J, Oswald O, Steuer N, Radonic A, Thulke S, Held TK, et al. Cytomegalovirus infections in allogeneic stem cell recipients after reduced-intensity or myeloablative conditioning assessed by quantitative PCR and pp65-antigenemia. Bone Marrow Transplant 2003;32:695-701.