ORIGINAL ARTICLE

The Role of Clinical, Therapeutic and Laboratory Findings in Monitoring of HCMV Infection in Bone Marrow Transplant Recipients

M Ramzi[1], R Yaghobi*[2], H Etminan[3]

[1]*Bone Marrow Transplant Unit and Haematology Research Center,* [2]*Shiraz Transplant Research Center, Namazi Hospital,* [3]*Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

Abstract

Background: Human cytomegalovirus (HCMV) has been an enormous threat for bone marrow transplant (BMT) recipients. For active and/or latent HCMV infection, diagnosis of the risk factors which increase the risk of post-transplant morbidity and mortality seems necessary. In this research, some of the HCMV risk factors were monitored and compared with HCMV molecular diagnostic methods for better detection of HCMV infection in BMT patients

Methods: HCMV risk factors including clinical, biological, biochemical, haematological indexes, and also anti-HCMV and transplant prophylactic and therapeutic conditioning regimens were monitored from March 2002 to March 2006, in 104 BMT patients referred to BMT Unit of Nemazee Hospital in Shiraz University of Medical Sciences and was compared with HCMV molecular methods for BMT donors and recipients' pre- and post-transplantation.

Results: Anti-HCMV-lgM was detected in 9.6% and 6.7% of BMT recipients and donors, respectively. Anti-HCMV-lgG was also detected in 8.7% and 9.1% of recipients and donors, pre-transplant, respectively. HCMV-PCR results were positive in 20% of recipients and 33.3% of donors. Significant correlations were observed between HCMV positive results and the use of a therapeutic dose, but not the prophylactic dose of glucocorticoids and cyclosporine, pre and post-transplantation. Fasting blood sugar, creatinine, globulin, and liver enzymes levels such as alkaline phosphates and asparagine transpherase significantly correlated with detection of HCMV-DNA in transplant patients. Also, negative results of HCMV-PCR significantly correlated with the use of prophylactic dose of acyclovir in BMT patients.

Conclusion: Significant correlations of positive and negative HCMV-PCR results with HCMV disease risk factors suggest the possible role of these factors on prognosis and monitoring of HCMV disease in BMT recipients pre- and post-transplantation.

Keywords: Risk factors; BMT; HCMV-PCR; Haematological indexes

Introduction

Human cytomegalovirus (HCMV) has been an enormous threat for BMT recipients and represents a major cause of morbidity and mortality after bone marrow transplantation.[1] Clinical studies have suggested a direct linkage between HCMV infection with clinically related symptoms with acceleration of the development of graft versus host disease (GVHD) and graft rejection.[1] In order to prevent and manage HCMV infections and/or its life threatening disorders, transplant recipients may benefit from three major therapeutic approaches including prophylaxis, pre-emptive therapy, and treatment of an established disease which linked directly with HCMV diagnostic procedures in BMT patients during the first months post-BMT.[1,2] Despite significant progress in diagnostic

***Correspondence:** Ramin Yaghobi, PhD, Shiraz Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-711-6474331, Fax: +98-711-6474331, e-mail: rayaviro@yahoo.com
Received: December 2, 2007 Accepted: September 12, 2008

and prevention strategies, HCMV infections exist in a dynamic environment and require constant reassessment of challenge in BMT recipients.[1,3] During this study in order to better define the impact of HCMV infection on BMT patients, some of the HCMV risk factors like clinical, biological, biochemical and haematological indexes, and also anti-HCMV, transplant prophylactic and therapeutic conditioning regimens, were monitored and then compared with HCMV-molecular methods.

Materials and Methods

From March 2002 to March 2006, 104 patients referred to BMT Unit of Namazee Hospital affiliated to Shiraz University of Medical Sciences in Shiraz, southern Iran, were enrolled in a retrospective study. Sixty five (62.5%) of these patients were male and 39 (37.5%) were female with the age range of 5-53 years old and mean of 19 years old. They were monitored during a 7 days pre-transplantation and 100 days post-transplantation period. Seven of these recipients underwent autologous transplantation and the remainder received bone marrow from allogenic HLA-identical sibling donors. All the clinical samples were tested with an in-house multiplex-PCR protocol for recipients and donors pre-transplantation and monitored weekly for 100 days in BMT patients post-transplantation.[4]

For monitoring of HCMV infection and/or disease, the following parameters as possible HCMV risk factors which increased the risk of post-transplant morbidity and mortality in BMT recipients, were analyzed. They include age and sex matching of donors and recipients, type of immunosuppressive therapy, type of underlying disease, kind of transplantation, type of relationship between donors and recipients, haematological and biochemical indexes of BMT recipients, type of symptoms and grade of GVHD, prophylactic and/or therapeutic dose of anti-GVHD drug regimen, HCMV IgM and IgG serological status of donors (D) and recipients (R) at the time of transplantation, HCMV-PCR results of donors and recipients pre and post-transplantation, HCMV clinical-like symptoms, and prophylactic and/or therapeutic dose of anti-HCMV drug regimens.

The patients underwent the antiviral and conditioning regimens according to standard protocol approved by institutional policy. HCMV-related active disease was defined according to Ljungman et al.[4]

The possible HCMV risk factors increasing the risk of post-transplant morbidity and mortality in BMT recipients were confronted to results of the CMV qualitative amplification by using Chi-square, two-tail Fisher's Exact test, association methods of SPSS for windows (version 15, Chicago, IL, USA).

Results

During the study period, the complete characteristic data of 104 BMT recipients were analyzed. The demographic data are summarized in Table 1. Donor and recipient sex match were detected in 28 (27.0%) male/male and in 23 (22.0%) female/female relationships. Donor and recipient sex mismatch was detected in 25 (24.0%) male/female and in 15 (14.5%) female/male relationships.

Table 1: Demographic data of BMT recipients and donors.

Variables	n (%)
Number of patients	104 (100.0)
Median age in years (range)	19 (r:5-53)
Sex	
Male	65 (62.5)
Female	39 (37.5)
Underlying disease	
Thalassemia major	42 (40.5)
Acute myelogenous leukemia	22 (21.2)
Chronic myelogenous leukemia	14 (13.5)
Acute lymphoblastic leukemia	7 (6.7)
Aplastic anemia	7 (6.7)
Hodgkin lymphoma	5 (4.8)
Non-Hodgkin lymphoma	3 (2.9)
Multiple myeloma	3 (2.9)
Ewing sarcoma	1 (1.0)
Type of transplantation	
Autologus	7 (6.7)
Allogen	97 (93.3)
Conditioning regimen	
Myleoablative	98 (94.2)
Non-myloeablative	6 (5.8)
Donor-recipient sex match	
Male/Male	25 (27.0)
Male/Female	25 (24.0)
Female/Male	15 (14.4)
Female/Female	23 (22.1)

The high prevalence of underlying diseases in BMT recipients was related to thalassemia major (40.5%), acute myelogenous leukemia (21.0%), and chronic myelogenous leukemia (13.5%), respectively. HCMV infection was diagnosed mostly in BMT candidates

with underlying diseases of acute myelogenous leukemia (59.0%), thalassemia major (55.0%), and chronic myelogenous leukemia (43.0%), respectively.

Nonmyeloablative conditioning regimen was preformed for only 5.8% of BMT patients and the rest received mayeloablative drug regimen.

Acute GVHD (aGVHD) which was categorized clinically to four types was diagnosed in 87.4% of BMT post-transplantation patients. Grade I of aGVHD was detected in 32.7% of BMT recipients and Grade I-IV of aGVHD in 61.5% of HCMV infected BMT patients (Table 2), but grading of aGVHD could not significantly increase the risk of HCMV infection post-BMT (P>0.05).

Table 2: Grading of acute GVHD in HCMV infected BMT patients.

Grade of acute GVHD	No. (%)	HCMV infection No. (%)
None	20 (19.2)	
Grade I	34 (32.7)	22 (65.0)
Grade II	16 (15.4)	8 (50.0)
Grade III	18 (17.3)	10 (56.0)
Grade IV	16 (15.4)	12 (75.0)

HCMV IgM and IgG serostatus were analyzed for donors and recipients. Positive results of HCMV IgM were detected in 7.7% of donors and 9.6% of recipients. HCMV IgG positive results were detected in 57.7% and 84.6% of donors and recipients, respectively. The overall pattern of HCMV IgM and IgG antibodies in BMT donors and recipients (D/R) is presented in Table 3.

HCMV genome was detected in 12.0% of donors by qualitative in house-HCMV-PCR pre-transplantation. HCMV-DNA was detected in 20.0% of BMT patient's pre-transplantation. Also, from the first to 10th week period, the positive results of HCMV-PCR in BMT recipients were 21.6%, 14.7%, 37.7%, 50.7%, 32.7%, 40.0%, 43.0%, 48.5%, 61.0%, and 57.0%, respectively.

The use of prophylactic dose of anti-GVHD drug regimen including glucocorticoids and cyclosporine and the use of therapeutic dose of cellcept were not significantly associated with detection of HCMV-DNA in blood samples of BMT recipients pre- and post-transplantation. Therapeutic dose of prednisolon and methyl prednisolon (*P=0.05*) and cyclosporine (*P=0.05*) in patients with different grades of GVHD clinical symptoms was significantly associated with positive results of HCMV-PCR in the second week period post-BMT.

Table 3: Pre-transplant donor and recipient HCMV serostatus.

Donor/recipient HCMV serostatus	n (%)
Recipient HCMV serostatus	
IgM Antibody	
Negative	87 (83.6)
Positive	10 (9.6)
Not tested	7 (6.7)
IgG Antibody	
Negative	9 (8.7)
Positive	88 (84.6)
Not tested	7 (6.7)
Donor HCMV serostatus	
IgM Antibody	
Negative	58 (55.8)
Positive	8 (7.7)
Not tested	38 (36.5)
IgG Antibody	
Negative	6 (5.7)
Positive	60 (57.7)
Not tested	38 (36.5)
Donor / recipient IgM serostatus	
D^+ / R^+	1 (1.0)
D^- / R^+	5 (4.8)
D^+ / R^-	6 (5.8)
D^- / R^-	51 (49.0)
Not tested	47 (45.2)
Donor / recipient IgG serostatus	
D^+ / R^+	54 (52.0)
D^+ / R^-	5 (4.8)
D^- / R^+	2 (1.9)
D^- / R^-	2 (1.9)
Not tested	47 (45.2)

Pre- and post-BMT use of intravenous immunoglobulin (IVIG) could not prevent HCMV disease and/or reactivation. But the use of acyclovir as an anti-herpetic agent was significantly associated with negative results of HCMV-PCR.

Weekly and blind (separate) monitoring of the efficiency of anti-HCMV therapy with gancyclovir by HCMV-PCR protocol showed that ganciclovir does not have a significant role in the control of HCMV disease.

Trombocytosis (P=0.02) and hemoglobinemia (P=0.05) may have a significant role in decreasing HCMV-PCR positive results. White blood cell count, sodium, potassium, bilirubin, albumin, and alanine transpherase levels were not significant causes of increase in HCMV infection in BMT patients. Statistical relationships were identified between fasting blood sugar (P=0.02), ceratinine (P=0.02), globulin (P=0.01), and liver enzyme levels like alkaline phosphates (P=0.05) and asparagine transpherase (P=0.05) with

detection of HCMV-DNA in transplant patients.

Discussion

The importance of HCMV as an opportunistic infection among immunocompromised patients, especially among BMT patients, was documented in numerous studies.[2,5] Human cytomegalovirus infection may lead to different clinical complications and may trigger the development of GVHD in transplant recipients.[2,6] Moreover, HCMV infection may induce an immunosuppressive effect and the development of other complications.[5] Therefore, it is critical to study different risk factors which may lead to a better definition of diagnostic methods, preventive strategies, and treatment protocols of HCMV infection in transplant patients.[2]

Among the demographic data, although most of patients are in paediatric age range (<19 years), age and gender were not considered as risk factors in developing HCMV reactivation or disease. However, it has been demonstrated that older age represents a risk factor for developing HCMV disease and for transplant-related mortality.[7,8]

Although the role of underlying disease has not been specifically studied as a risk factor of HCMV reactivation or disease development after allogeneic or autologous BMT, it has been demonstrated that the underlying disease stage is a highly significant predictor of HCMV disease related mortality in BMT patients. However, diagnosis of chronic myelogenous leukemia could represent a negative prognostic factor in patients receiving T cells depleted bone marrow from HCMV positive donors.[7,9] Similarly, without any significant associations, most of HCMV infected patients had underlying diseases such as acute myelogenous leukemia (59.0%), thalassemia major (57.0%), and chronic myelogenous leukemia (43.0%).

Post-transplant development of graft-versus-host disease (GVHD) is another risk factor of HCMV reactivation or disease in BMT patients.[6] In this study, 87.4% of BMT patients suffered from grade I-IV of aGVHD. Although HCMV infection was seen in 50-75% of transplant recipients with clinical symptoms of aGVHD, significant relationships were not defined between aGVHD and onset of post-transplant HCMV infection. In different studies, HCMV infection did not elevate the incidence of aGVHD,[10-12] but in another, aGVHD significantly increased the risk of HCMV infection.[7,13]

HCMV serologic status is one of the key risk factors, especially in unrelated donor transplant recipients.[9,14-18] HCMV-seropositive patients have poor outcome than seronegative recipients despite improvement in preventive strategies against HCMV disease such as antiviral prophylaxis and preemptive therapy.[9,14-18] The pattern of HCMV serostatus has shown the low and high prevalence of HCMV-IgM and HCMV-IgG antibodies in both BMT donors and recipients, respectively (Table 3). The lowest HCMV-IgM seroprevalence (1.0%), and reversely, the highest HCMV-IgG seroprevalence (52.0%) belonged to D^+/R^+ relationships. But, the highest HCMV-IgM seroprevalence (49.0%), and reversely, the lowest HCMV-IgG seroprevalence (2.0%) belonged to D^-/R^- HCMV serology relationships. D^+/R^- HCMV relationship, which mostly threatened the post-BMT healthy conditions, was only 6.0% and 5.0% for HCMV-IgM and HCMV-IgG serostatus of donors and recipients, respectively.

Different types of PCR protocols were run for detection and monitoring of HCMV infection in transplant patients, especially BMT recipients.[3,10] In this study, the HCMV infection was detected and monitored in donors and recipients by two sensitive and specific qualitative multiplex-PCR methods pre- and post-BMT. The rise of HCMV infection was initiated from the third week (37.7%) and continued to the 10th week (57.0%) post-transplantation.

Monitoring of HCMV infection and/or disease by this HCMV-PCR protocol was compared with different risk factors of transplant patients. One of these HCMV risk factors is transplant conditioning regimens. Myeloablative conditioning regimens seek to destroy the existing cells, allowing for replacement with the graft cells. Mixed chimerism occurs with coexistence of host and donor cells until the replacement of graft material prevails in patients using non-myeloablative conditioning regimen.[3] In this study, prophylactic dose unlike therapeutic dose of anti-GVHD drug regimen was not significantly associated with detection of HCMV-DNA in BMT recipients pre-transplantation.

IVIG could not prevent HCMV disease and/or reactivation in BMT patients. Similarly, in two separate randomized, controlled trials of treatment of seronegative recipients of allogeneic BMT, use of HCMV-IVIG for viral prophylaxis showed no difference in disease incidence.[19,20] In other studies, IVIG or HCMV immunoglobulin failed to show either consistent positive results for HCMV-related complications or survival benefits.[21-24] Overall, antibody treatments are not currently recommended for HCMV prophylaxis[25] and uncertainty remains over the usefulness

of IVIG or hyperimmune globulin for the prevention of non-HCMV complications.[3]

Acyclovir administration as an anti-HCMV prophylactic agent was significantly associated with a decrease in the rate of HCMV infection. Similarly, the first trial use of antiviral prophylaxis of intravenously high-dose acyclovir from 5 days before engraftment to 30 days post-transplantation in seropositive allogeneic bone marrow transplant recipients showed the reduction of the risk of HCMV infection and invasive disease.[3,26] With the apparent success of acyclovir, a more extensive prospective, double-blind trial showed a survival advantage for the IV acyclovir/oral acyclovir group compared with the controls.[27,28]

Monitoring of anti-HCMV ganciclovir therapy by qualitative PCR protocol did not significantly reduce the incidence of HCMV disease post-BMT. Similarly, ganciclovir has also been used as HCMV prophylaxis but no survival advantage was demonstrated and severe neutropenia was observed in all studies.[27,29] But, the results of early prophylaxis with ganciclovir in seropositive allogeneic bone marrow transplant recipients have been reported from 3 randomized, double-blind studies with slightly different protocols. All these studies demonstrated a significant (P=0.001) reduction of HCMV infection or disease during the first 100 day period post-transplantation.[27,30,31]

The significant role of some reported haematological and biochemical indexes in the increase and decrease of HCMV-PCR positive results demonstrated the need of further and thorough research about the exact role of these demographic data in prognosis of HCMV disease in BMT patients. Also in another study, no statistically significant difference was observed when haematological parameters were studied.[2]

The study of these risk factors, which increase the risk of post-transplant morbidity and mortality, in monitoring of HCMV infection and/or disease is beneficial. However, there is a need for more thorough studies to define and confirm the role of these indexes, especially haematological and biochemical factors, in management of HCMV disease in pre- and post-BMT conditions.

Acknowledgment

We would like to thank the Office of Vice Chancellor for Research of Shiraz University of Medical Sciences for financial support and the Center for Development of Clinical Studies of Nemazee Hospital for editorial assistance.

Conflict of interest: None declared.

References

1 Landolfo S, Gariglio M, Gribaudo G, Lembo D. The human cytomegalovirus. *Pharmacol Ther* 2003;**98(3)**:269-97. [12782241] [doi:10.1016/S0163-7258(03)00034-2]

2 Kamar N, Mengelle C, Yahyaoui S, Sandres-Sauné K, Durand D, Izopet J, Rostaing L. Follow-up of 28 HCMV seropositive renal-transplant recipients: comparison of clinical, biological and virological parameters in the groups of treated versus untreated infected patients. *J Clin Virol* 2005;**33(1)**:*35-42.* [15797363] [doi:10.1016/j.jcv.2004.09.028]

3 Boeckh M, Nichols G, Papanicolaou G, Rubin R, Wingard J R, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges and future strategies. *Biol Blood Marrow Transplant* 2003;**9(9)**:543-58. [14506657] [doi:10.1016/S1083-8791(03)00287-8]

4 Yaghobi R, Behzad-Behbahani A, Sabahi F, Roustaee MH, Alborzi A, Ramzi M, Nourani H. Comparative analysis of double primer PCR assay with plasma and leukocytes and antigenemia for diagnosis of active human cytomegalovirus infection in bone marrow transplant patients. *Bone Marrow Transplant* 2005;**35(6)**:595-9. [15665849] [doi:10.1038/sj.bmt.1704797]

5 Patel R, Paya CV. Infections in solid-organ transplant recipients. *Clin Microbiol Rev* 1997;**10(1)**:86-124. [8993860]

6 Soderberg-Naucler C, Emery VC. Viral infections and their impact on chronic renal allograft dysfunction. *Transplantation* 2001;**71**:24-30. [11583485]

7 Castagnola E, Cappelli B, Erba D, Rabagliati A, Lanino E, Dini G. Cytomegalovirus infection after bone marrow transplantation in Children. *Hum Immunol* 2004;**65(5)**:416-22. [15172440] [doi:10.1016/j.humimm.2004.02.013]

8 Ljungman P, Aschan J, Lewensohn-Fuchs I, Carlens S, Larsson K, Lönnqvist B, Mattsson J, Sparrelid E, Winiarski J, Ringdén O. Results of different strategies for reducing cytomegalovirus-associated mortality in allogeneic stem cell transplant recipients. *Transplantation* 1998;**66(10)**:1330-4. [9846518] [doi:10.1097/00007890-199811270-00012]

9 Ljungman P, Brand R, Einsele H, Frassoni F, Niederwieser D, Cordonnier C. Donor CMV serologic status and outcome of CMV-seropositive recipients after unrelated donor stem cell transplantation: an EBMT mega file analysis. *Blood* 2003 15;**102(13)**:4255-60. [12933590]

10 Hazar V, Ugur A, Colak D, Saba R, Tezcan G, Kupesiz A, Karadogan I, Gultekin M, Yesilipek A, Undar L. Cytomegalovirus antigenemia and outcomes of patients undergoing allogenic peripheral blood stem cell transplantation: effect of long term high-dose acyclovir prophylaxis and preemptive gancilovir treatment. *Jpn J Infect Dis* 2006;**59(4)**:216-21. [16936338]

11 Ottinger HD, Beelen DW, Schaefer UW, Grosse-Wilde H. Improved immuno reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. *Blood* 1996;**88(7)**:2775-9. [8839875]

12 Trenschel R, Ross S, Husing J, Ottinger H, Elmaagacli A, Roggendorf M, Schaefer UW, Runde V. Reducing risk of persisting cytomegalovirus pp65 antigenemia and cytomegalovirus interstitial pneumonia following allogenic PBSCT. *Bone Marrow Transplant* 2000;**25(6)**:665-72. [10734302] [doi:10.1038/sj.bmt.1702216]

13 Matthes-Martin S, Aberle SW, Peters C, Holter W, Popow-Kraupp T, Pötschger U, Fritsch G, Ladenstein R, Rosenmayer A, Dieckmann K, Gadner H. CMV-viraemia during allogenic bone marrow transplantation in paediatric patients: association with survival and graft-versus-host disease. *Bone Marrow Transplant* 1998;**21**:S53-6. [9630327]

14 Broers AE, van Der Holt R, van Esser JW, Gratama JW, Henzen-Logmans S, Kuenen-Boumeester V, Löwenberg B, Cornelissen JJ. Increased transplant-related morbidity and mortality in CMV-seropositive patients despite highly effective prevention of CMV disease after allogeneic T-cell-depleted stem cell transplantation. *Blood* 2000;**95(7)**:2240-5. [10733491]

15 Meyers JD, Flournoy N, Thomas ED. Risk factors for cytomegalovirus infection after human marrow transplantation. *J Infect Dis* 1986;**153(3)**:478-88. [3005424]

16 Bacigalupo A, Tedone E, Sanna MA, Moro F, Van Lint MT, Grazi G, Balestreri M, Frassoni F, Occhini D, Gualandi F. CMV infections following allogeneic BMT: risk factors, early treatment and correlation with transplant related mortality. *Haematologica* 1992;**77(6)**:507-13. [1337746]

17 Takenaka K, Gondo H, Tanimoto K, Nagafuji K, Fujisaki T, Mizuno S, Miyamoto T, Okamura T, Hayashi S, Eto T, Osaki K, Yamasaki K, Shibuya T, Harada N, Teshima T, Matsuishi E, Minematsu T, Minamishima Y, Harada M, Niho Y. Increased incidence of cytomegalovirus (CMV) infection and CMV-associated disease after allogeneic bone marrow transplantation from unrelated donors. The Fukuoka Bone Marrow Transplantation Group. *Bone Marrow Transplant* 1997;**19(3)**:241-8. [9028553] [doi:10.1038/sj.bmt.170 0637]

18 Craddock C, Szydlo RM, Dazzi F, Olavarria E, Cwynarski K, Yong A, Brookes P, de la Fuente J, Kanfer E, Apperley JF, Goldman JM. Cytomegalovirus seropositivity adversely influences outcome after T-depleted unrelated donor transplant in patients with chronic myeloid leukaemia: the case for tailored graft-versus-host disease prophylaxis. *Br J Haematol* 2001;**112(1)**:228-36. [11167809] [doi:10.1046/j.1365-2141.2001.02519.x]

19 Bowden RA, Fisher LD, Rogers K, Cays M, Meyers JD. Cytomegalovirus (CMV)-specific intravenous immunoglobulin for the prevention of primary CMV infection and disease after marrow transplant. *J Infect Dis* 1991;**164(3)**:483-7. [1651360]

20 Ruutu T, Ljungman P, Brinch L, Lenhoff S, Lönnqvist B, Ringdén O, Ruutu P, Volin L, Albrechtsen D, Sallerfors B, Ebeling F, Myllylä G. No prevention of cytomegalovirus infection by anti-cytomegalovirus hyperimmune globulin in seronegative bone marrow transplant recipients. The Nordic BMT Group. *Bone Marrow Transplant* 1997;**19(3)**:233-6. [9028551] [doi:10.1038/sj.bmt.1700649]

21 Bass EB, Powe NR, Goodman SN, Graziano SL, Griffiths RI, Kickler TS, Wingard JR. Efficacy of immune globulin in preventing complications of bone marrow transplantation: a meta-analysis. *Bone Marrow Transplant* 1993;**12(3)**:273-82. [8241987]

22 Guglielmo BJ, Wong-Beringer A, Linker CA. Immune globulin therapy in allogeneic bone marrow transplant: a critical review. *Bone Marrow Transplant* 1994;**13(5)**:499-510. [8054903]

23 Messori A, Rampazzo R, Scroccaro G, Martini N. Efficacy of hyperimmune anti-cytomegalovirus immunoglobulins for the prevention of cytomegalovirus infection in recipients of allogeneic bone marrow transplantation: a meta-analysis. *Bone Marrow Transplant* 1994;**13(2)**:163-7. [8205085]

24 Zikos P, Van Lint MT, Lamparelli T, Gualandi F, Occhini D, Mordini N, Berisso G, Bregante S, Bacigalupo A. A randomized trial of high dose polyvalent intravenous immunoglobulin (HDIgG) vs. cytomegalovirus (CMV) hyperimmune IgG in allogeneic hemopoietic stem cell transplants (HSCT). *Haematologica* 1998;**83(2)**:132-7. [9549924]

25 Centers for Disease Control and Prevention; Infectious Diseases Society of America; American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2000;**6(6a)**:659-713; 715;717-27. [11185897]

26 Meyers JD. Prevention and treatment of cytomegalovirus infection after marrow transplantation. *Bone Marrow Transplant* 1988;**3(2)**:95-104. [2844342]

27 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(10)**:4063-71. [8916975]

28 Prentice HG, Gluckman E, Powles RL, Ljungman P, Milpied NJ, Camara R, Mandelli F, Kho P, Kennedy L, Bell AR. Long-term survival in allogeneic bone marrow transplant recipients following acyclovir prophylaxis for CMV infection. The European Acyclovir for CMV Prophylaxis Study Group. *Bone Marrow Transplant* 1997;**19(2)**:129-33. [9116609] [doi:10.1038/sj.bmt.1700498]

29 Salzberger B, Bowden RA, Hackman RC, Davis C, Boeckh M. Neutropenia in allogeneic marrow transplant recipients receiving ganciclovir for prevention of cytomegalovirus disease: risk factors and outcome. *Blood* 1997;**90(6)**:2502-8. [9310503]

30 Goodrich JM, Bowden RA, Fisher L, Keller C, Schoch G, Meyers JD. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. *Ann Intern Med* 1993;**118(3)**:173-8. [8380242]

31 **31** Winston DJ, Ho WG, Bartoni K, Du Mond C, Ebeling DF, Buhles WC, Champlin RE. Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients. Results of a placebo-controlled, double-blind trial. *Ann Intern Med* 1993;**118(3)**:179-84. [8380243]