Brief Original Article

Cytomegalovirus reactivation following hematopoietic stem cell transplantation

Sanjeev Kumar Sharma[1], Suman Kumar[1], Narendra Agrawal[1], Lavleen Singh[2], Anjan Mukherjee[3], Tulika Seth[1], Pravas Mishra[1], Sandeep Mathur[2], Lalit Dar[3], Manoranjan Mahapatra[1]

[1] *Department of Hematology, All India Institute of Medical Sciences, New Delhi, India*
[2] *Department of Pathology All India Institute of Medical Sciences, New Delhi, India*
[3] *Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India*

Abstract

Introduction: There is a high prevalence of cytomegalovirus (CMV) seropositivity in developing countries. An apparent risk of CMV reactivation increases following hematopoeitic stem cell transplantation. With effective surveillance and timely treatment using anti-viral therapy, morbidity and mortality associated with CMV reactivation can be reduced.

Objectives: To evaluate the incidence and morbidity associated with CMV reactivation following hematopoeitic stem cell transplantation.

Methodology: We retrospectively analysed 136 hematopoeitic stem cell transplant recipients at our centre for CMV reactivation and their complications. Quantification of CMV-DNA was done by PCR. CMV disease was confirmed histologically via CMV inclusion bodies or immunostaining of biopsy of the affected organ, mainly the gastrointestinal tract.

Results: A total of 13 out of 136 patients (9.56%) had CMV reactivation. 6 out of 13 patients had CMV disease, 3 of which died (23.1% of patients with CMV reactivation). CMV reactivation occurred at a median duration of 52.5 days post transplantation (range 35-178 days). The gastrointestinal tract was the organ most commonly affected by CMV. The median follow-up was 14 months (range 6 - 64 months).

Conclusion: Through a higher rate of sero-prevalance in developing countries, the incidence of CMV infection following hematopoeitic stem cell transplantation is comparable to that reported in Western literature. Oral valganciclovir was an effective pre-emptive therapy for CMV disease.

Key words: Cytomegalovirus; Stem cell transplantation; Ganciclovir

J Infect Dev Ctries 2013; 7(12):1003-1007. doi:10.3855/jidc.2947

(Received 23 August 2012 – Accepted 29 October 2013)

Introduction

Infections are a major cause of morbidity and mortality following hematopoeitic stem cell transplantation for various malignant and non-malignant hematological diseases. Risk of infection is particularly higher in developing countries where endemic diseases are also a major contributor to infections during immunocompromised state following hematopoietic stem cell transplantation (HSCT). Though the incidence of CMV disease during the first year after HSCT has decreased from approximately 30%, in the period before ganciclovir, to 8%, CMV reactivation is still a common complication in HSCT recipients. Its early recognition and pre-emptive treatment can prevent the high mortality associated with CMV disease. The number of patients undergoing HSCT has increased in developing countries but data is scarce on CMV reactivation following such procedures. Moreover, multiple co-infecting pathogens including hepatitis viruses, dengue, tuberculosis, opportunistic fungal pathogens, pneumocystis and other herpes viruses can confound the clinical presentation [1-3]. We analysed the incidence of CMV reactivation and associated morbidity in patients following stem cell transplantation.

Methodology

The study was conducted within the Department of Hematology, All India Institute of Medical Sciences, New Delhi, India. 136 patients who underwent HSCT for various hematological diseases were analysed retrospectively for CMV reactivation. The conditioning regimen used for HSCT included fludarabine (30mg/m^2 for 6 days), cyclophosphamide (60mg/kg/day for 2 days) and rabbit antithymocyte globulin (3.5 mg/kg/day for 2 days) in patients with aplastic anemia; and busulfan (3.2 mg/kg/day for 4

days) cyclophosphamide (60 mg/kg/day for 2 days) in the other patients (Table 1).

Quantification of plasma CMV-DNA was done by Quantitative real-time polymerase chain reaction (qPCR) assays, which have been found to be equally effective as whole blood PCR [4-5]. Of the CMV-DNA copies, more than 1000/ml were considered as suggestive of CMV reactivation. CMV infection was defined as detection of CMV-DNA in the plasma by PCR without involvement of any organ, whereas CMV disease was identified by histological confirmation of CMV inclusion bodies or immunostaining of the biopsy specimen. Patients with CMV reactivation were treated with intravenous ganciclovir (5mg/kg twice daily) or oral valganciclovir (900mg twice daily). The quantification of CMV-DNA titers was done every 15 days till two consecutive results were negative.

Results

A total of 136 patients underwent HSCT at our institute over the last 7 years. The most common indications for allogeneic sibling matched transplantations were aplastic anemia (41.2%), followed by acute myeloid leukemia (14.7%). Median patient age, who underwent SCT, was 25 years, with a male to female ratio of 3:1. Grade 2-4 acute graft versus host disease (GvHD) occurred in 30.14% patients.

Out of the 136 patients who underwent HSCT, 13 patients (9.5%) developed CMV reactivation. Table 1 shows the clinical characteristics of the patients and table 2 shows the detailed description of the patients who developed CMV reactivation. Median age of patients, with CMV reactivation, was 27 years (range 9-47 years) with a male to female ratio of 3:1. The median blood transfusions received before HSCT were 20 units (0-52 units). The neutrophil engraftment in these patients had occurred at a median of 10 days (8-15 days). All donors and recipients were positive for CMV-IgG, except for one donor who was CMV-IgG negative and a recipient being CMV-IgG positive. CMV-IgM was negative in all donors and recipients. CMV reactivation occurred at a median duration of 53 days (range 35-178 days) post transplant. CMV-DNA PCR titers ranged from 1,500 to 6.5 x 10^5 copies/ml (median 30,000 copies/ml). The average number of samples collected per patient were 7 (range 5-11). Of the patients who developed CMV reactivation following HSCT 9 patients had severe aplastic anemia, and 1 patient had acute myeloid leukemia, chronic myeloid leukemia-accelerated phase, myelodysplastic syndrome (MDS RAEB-1), and pure red cell aplasia (Table 2). 12 of these patients had undergone peripheral blood stem cell transplantation (PBSCT) and 1 had undergone bone marrow transplant (BMT), all transplants were HLA matched sibling transplants. CMV reactivation was found in 6 patients on routine evaluation, while 5 patients had diarrhea and 1 patient had vomiting and cytopenias at the time of CMV reactivation. CMV enteritis was proven by histopathological examination of the gut biopsy specimen.

Patients with suspected CMV disease (who presented with diarrhea and esophagitis) had been treated with intravenous gancyclovir 5mg/kg twice daily. Patients who had CMV infection without disease had been treated with oral valganciclovir 900mg, twice daily for 2 weeks, followed by once daily until two reports of CMV-DNA PCR turned negative. Two patients were receiving steroids for acute gut and skin GvHD respectively, at the time of CMV reactivation. All the patients with CMV infection had recovered, whereas 3 out of 6 patients with CMV disease died. One of these patients had ganciclovir resistant CMV disease and died of pneumonitis and encephalitis. The median total leukocyte count (TLC) and the median absolute lymphocyte count (ALC) at the time of CMV infection were $2.88x10^9$/l (range $1.0\text{-}6.5x10^9$/l) and $0.71x10^9$/l ($0.21\text{-}1.03x10^9$/l) respectively. ALC of the patients who had CMV infection was $0.7x10^9$/l and those who developed CMV disease was $0.64x10^9$/l (p=0.79). The median followup was 14 months (range 6 - 64 months).

Discussion

In developing countries, there is a high CMV seroprevalance in the community. The risk is further increased by limited facilities of leuko-depleted blood products available for the multi-transfused patients with hematological diseases. Seroprevalance in India is very high with about 95% of the healthy blood donors in India being CMV seropositive [6-7]. Steroid therapy, GvHD and lack of CMV-specific T cells post-transplant are major risk factors for CMV reactivation. Two of our patients were on steroids for acute GvHD at the time of CMV reactivation and four patients who had presented with acute onset diarrhea were found to have raised CMV-DNA titers (>1000 copies/ml) without gut biopsy findings typical of CMV enteritis and were treated with intravenous ganciclovir along with intravenous methylprednisolone suspecting gut GvHD.

Table 1. Characteristic features of patients who developed CMV infection following stem cell transplantation (n =13)

Median age, years (range)	27 (9-47)
Male to female ratio	3:1
Median follow-up, months (range)	14 (6-64)
Diseases	
Aplastic anaemia	9
AML	1
CML	1
MDS	1
PRCA	1
Conditioning regimen used	
Flu/Cy/ATG	9
Bu/Cy	4
Transplant	
PBSCT	12
BMT	1
Median CD 34+ cell dose/kg	6.31 x 10^6
Median neutrophil engraftment, days	10
Pre-transplant median RBC transfusions received	20
Median ALC at the time of CMV reactivation/µl	0.71x10^9
Clinical presentation	
Asymptomatic (routine evaluation)	6
Diarrhea	5
Vomitings	1
Cytopenia	1
Patients on steroids at the time of CMV reactivation	2
Treatment used	
Gancyclovir (i/v)	8
Valganciclovir (oral)	5
Mortality (%)	3 (23.1)

(AML acute myeloid leukemia, CML chronic myeloid leukemia, MDS myelodysplastic syndrome, PRCA pure red cell aplasia, ALC absolute lymphocyte count, PBSCT peripheral blood stem cell transplantation, BMT bone marrow transplantation)

Table 2. Detailed description of the thirteen patients with CMV reactivation following hematopoietic stem cell transplantation

S.No.	Age (years)	Sex	Diagnosis	Days post HSCT CMV detected	Clinical presentation	CMV-DNA titer (copies/ml)	GvHD	Outcome
1	15	M	AA	59	Routine evaluation	5x10^3	None	Recovered
2	35	M	AA	42	Diarrhea	1.5x10^3	None	Recovered
3	45	F	AA	49	Routine evaluation	1.16x10^5	None	Recovered
4	31	M	PRCA	42	Cytopenias	4.5x10^4	None	Recovered
5	22	M	AA	56	Routine evaluation	1x10^4	None	Recovered
6	21	M	AA	48	Routine evaluation	2.2x10^3	None	Recovered
7	28	M	AA	62	Diarrhea	6.5x10^5	Gut	Recovered
8	10	M	AA	42	Routine evaluation	8.9x10^4	Gut	Recovered
9	9	F	AA	35	Diarrhea	2x10^4	Gut	Recovered
10	43	M	CML-AP	135	Diarrhea	3x10^4	Gut	Expired
11	47	M	MDS-RAEB 1	42	Diarrhea	1x10^5	Gut, Skin	Expired
12	26	F	AML-M4	112	Vomiting	3x10^4	Skin	Expired
13	27	M	AA	60	Routine evaluation	7.5x10^4	None	Recovered

(CMV- cytomegalovirus, HSCT- hematopoeitic stem cell transplantation, AA-aplastic anemia, PRCA- pure red cell aplasia, CML-chronic myeloid leukemia-accelerated phase, MDS-myelodysplastic syndrome, AML- acute myeloid leukemia)

The manifestations of CMV disease may include hepatitis, gastrointestinal ulceration, pneumonitis, retinitis or central nervous system disease [8]. Quantitative real-time polymerase chain reaction (qPCR) assays for CMV-DNA are highly sensitive [4-5]. PCR can be positive, even if antigenemia is negative. Disease may also occur because of missed surveillance tests or surveillance tests that are done too far apart [8]. Severely immunosuppressed patients have a rapid replication of CMV leading to increased morbidity and mortality seen following HSCT, particularly in those patients receiving steroids or having acute GvHD [10].

The threshold for treatment was 1000/ml copies of CMV-DNA or a more than 5-fold increase over baseline when lower DNA levels are detected [9]. The diagnosis of CMV gastrointestinal disease relies on detection of CMV in biopsy specimens by culture, immunohistochemistry, or detection of inclusion bodies. In a study by van Burik et al [11], the incidence of CMV enteritis at 2 years following HSCT averaged 2% over the 11.5-year study interval in 2240 patients. The median diagnosis time of CMV enteritis after HSCT was 91 days (range, 17-527 days). The overall survival rate was 35% at 2 years following the onset of enteritis. Five of our patients with raised CMV-DNA titers presented with diarrhea and colonic biopsy in 4 of them showed cytopathological evidence of CMV.

The pre-emptive treatment strategy is highly effective at managing CMV infection; CMV infections result from primary infection or reactivation, and most cases occur between 6 and 12 weeks after transplant [12-13]. In our study, CMV reactivation occurred in median duration of 52.5 days (range 35-178 days) post transplant. The current pre-emptive therapy with intravenous ganciclovir has decreased the incidence of CMV disease to rates ranging from 5% to 10.5% before day 100 after engraftment [14]. Our patients with CMV disease (who presented with diarrhea and esophagitis) had been treated with intravenous gancyclovir 5mg/kg twice daily for 2 weeks and patients who had CMV infection without disease had received oral valganciclovir 900mg twice daily for 2 weeks followed by once daily till CMV-DNA PCR turned negative.

Valganciclovir can be used in pre-emptive strategies of CMV disease in HSCT recipients because of oral administration, good bioavailability and low toxicity profile. Oral valganciclovir is a safe and effective alternative to intravenous ganciclovir as preemptive therapy for CMV reactivation in HSCT recipients [15,16]. Furthermore, oral therapy allowed the early initiation of treatment in most patients obviating the delay associated with the admission to the hospital to initiate intravenous therapy, particularly in developing countries where resources are limited. But, for patients with CMV enteritis intravenous ganciclovir was preferred.

Conclusion

In spite of high seroprevalance in our community, incidence of CMV reactivation is comparable to that reported in Western literature. There is a lack of facilities of leukodepleted blood products in majority of hospitals in developing countries and weekly CMV-DNA PCR monitoring and in-patient pre-emptive treatment is not cost-effective. Patients with CMV reactivation without active disease can be easily managed with oral valganciclovir with once fortnightly CMV-DNA monitoring, without need for admission and intravenous ganciclovir with good outcome and is cost effective. Management of CMV enteritis requires admission and treatment with intravenous ganciclovir.

Acknowledgements

We are thankful to Dr Sandeep Sharma and Aashna Sharma for formatting the manuscript.

References

1. George B, Mathews V, Srivastava A, Chandy M (2004) Infections among allogeneic bone marrow transplant recipients in India. Bone Marrow Transplantation 33:311-315.
2. Sharma SK, Seth T, Mishra P, Gupta N, Agrawal N, Broor S, Mahapatra M, Saxena R (2011) Clinical profile of dengue infection in patients with hematological diseases. Mediterr J Hematol Infect Dis 3(1) e2011039.
3. Chakravarti A, Kashyap B, Matlani M (2009) Cytomegalovirus infection: an Indian perspective. Indian J Med Microbiol 27: 3-11.
4. Hebart H, Müller C, Löffler J, Jahn G, Einsele H (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.
5. Lisboa LF, Asberg A, Kumar D, Pang X, Hartmann A, Preiksaitis JK, Pescovitz MD, Rollag H, Jardine AG, Humar A (2011) The clinical utility of whole blood versus plasma cytomegalovirus viral load assays for monitoring therapeutic response. Transplantation 27: 231-236.
6. Kothari A, Ramachandran VG, Gupta P, Singh B, Talwar V (2002) Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. J Health Popul Nutr 20: 348-351.
7. Kumar H, Gupta PK, Kumar S, Sarkar RS (2008) Is seroprevalance of anti-IGM CMV among blood donors relevant in India? Indian J Pathol Microbiol 51: 351-352.
8. Ljungman P (2008) CMV infections after hematopoietic stem cell transplantation. Bone Marrow Transplant 42: 70–72.

9. Boeckh M and Ljungman P (2009) How we treat cytomegalovirus in hematopoietic cell transplant Recipients. Blood 113: 5711-5719.
10. Spector SA, Hsia K, Crager M, Pilcher M, Cabral S, Stempien MJ (1999) Cytomegalovirus (CMV) DNA load is an independent predictor of CMV disease and survival in advanced AIDS. J Virol 73: 7027-7030.
11. van Burik JH, Lawatsch EJ, DeFor TE, Weisdorf DJ (2001) Cytomegalovirus enteritis among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant 7: 674-679.
12. Machado CM, Dulley FL, Boas LS, Castelli JB, Macedo MC, Silva RL, Pallota R, Saboya RS, Pannuti CS (2000) CMV pneumonia in allogeneic BMT recipients undergoing early treatment of preemptive ganciclovir therapy. Bone Marrow Transplant 26: 413-417.
13. Boeckh M, Boivin G (1998) Quantitation of cytomegalovirus: methodologic aspects and clinical applications. Clin Microbiol Rev 11: 533-554.
14. Boeckh M,Nichols G,Papanicolaou G,Rubin R, Wingard JR, Zaia J (2003) Cytomegalovirus in hematopoietic stem celltransplant recipients: Current status, knownchallenges, and future strategies. Biol Blood Marrow Transplant 9: 543-558.
15. Ayala E, Greene J, Sandin R, Perkins J, Field T, Tate C, Fields KK, Goldstein S (2006) Valganciclovir is safe and effective as pre-emptive therapy for CMV infection in allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 37: 851-856.
16. Einsele H, Reusser P, Bornhauser M, Kalhs P, Ehninger G, Hebart H, Chalandon Y, Kröger N, Hertenstein B, Rohde F (2006) Oral valganciclovir leads to higher exposure to ganciclovir than intravenous ganciclovir in patients following allogeneic stem cell transplantation. Blood 107: 3002-3008.

Corresponding author

ManoranjanMahapatra, MD
Department of Hematology,
All India Institute of Medical Sciences,
New Delhi, India.
Email: mrmahapatra@hotmail.com
Phone: 91-9868397235

Conflict of interests: No conflict of interests is declared.