RESEARCH PAPER

Cytomegalovirus Infection as a Cause of Cytopenia After Chemotherapy for Hematological Malignancies

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Received on: September 22, 2011; Initial review: November 01, 2011; Accepted: April 18, 2012.

Objective: Unlike hematopoietic stem cell transplantation, there is very little information on cytomegalovirus (CMV) related cytopenias occurring in patients having acute lymphoblastic leukemia (ALL) or Non Hodgkin's lymphoma (NHL) receiving standard dose chemotherapy (SDCT). We studied the role of CMV infection in cytopenias after SDCT for childhood ALL or NHL.

Design: Retrospective study.

Setting: Pediatric Oncology Unit.

Methods: Between January 2007 and March 2010, we screened all children having ALL/ NHL having prolonged cytopenia (ANC<1,000/cmm and/or platelets <1,00,000/cmm; >10 days beyond date for next chemotherapy; not explainable on basis of previously administered chemotherapy) for CMV infection. Testing for CMV infection was done by pp65 antigen assay, qualitative or quantitative RT-PCR. CMV positive episodes were analyzed for relationship to previous chemotherapy, clinical features and response to treatment.

Results: As defined, 24 episodes of cytopenia were identified. CMV infection was detected in 13/24 (54%) episodes in 9 patients. Duration of cytopenia in patients having CMV infection: 14-126 days (median 28 d). Neutropenia or thrombocytopenia were seen in 11/13 and 13/13 episodes, respectively. Fever (2-20 d) and loose motions (3-60 d) in 11/13 and 9/13 episodes, respectively. Eye examination records were available in 5 children; 3 had simultaneous or delayed chorioretinitis. Gancyclovir was used in all but 1 CMV-positive episode. In treated cases, counts recovered after a median of 8 days (3-56 d).

Conclusion: Following chemotherapy for ALL/NHL, cytopenia that is prolonged or not explainable on the basis of chemotherapy toxicity should be evaluated for CMV infection.

Key words: *Chemotherapy, Cytomegalovirus, Cytopenia, Leukemia*.

Published online: 2012, August 05. PII: S-97475591100788-1

Cytomegalovirus (CMV) infection is a well recognized cause of pancytopenia in patients of hemato-lymphoid malignancies undergoing hematopoietic stem cell transplantation (HSCT) [1-5]. However, there is very little information on similar problems occurring in patients of hemato-lymphoid malignancies receiving standard dose chemotherapy [6-9]. Prolonged cytopenias may occur in ALL/NHL therapy due to poor disease response, chemotherapy toxicity or viral infections. These could delay chemotherapy and increase the infection related morbidity or mortality. We present our experience regarding CMV infection causing prolonged cytopenias after standard dose chemotherapy in children having acute lymphoblastic leukemia (ALL) or Non-Hodgkin's lymphoma (NHL).

METHODS

Since January 2007, it has been our protocol to check for CMV infection in patients receiving standard chemotherapy for ALL or NHL who develop prolonged cytopenia (platelet count <1,00,000/mm^3 and/or absolute neutrophil count (ANC) <1,000/mm^3). We had screened children <15 years of age, receiving standard chemotherapy for ALL/NHL with cytopenia persisting >10 days beyond the scheduled date of next chemotherapy.

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We retrospectively analyzed case records of all eligible children between January 2007 and March 2010. Data regarding CMV testing, relationship to previous chemotherapy, clinical features and response to gancyclovir treatment were noted. Total duration of cytopenia was measured from the day of first detection of the cytopenia till recovery of platelet count > 1,00,000/mm^3 and ANC > 1,000/mm^3.

CMV testing was not done in cases where cytopenias occurred during ALL induction and those which could be

directly attributed to chemotherapy or other drug toxicity (mercaptopurine, linezolid, etc.). Patients having cytopenia >10 days, but showing an improving trend in the cytopenia were also not tested for CMV.

All patients with ALL were treated with 4 drug induction (vincristine, L-asparginase, prednisolone, daunomycin, intrathecal methotrexate) followed by consolidation using moderate dose methotrexate and/or high dose cytarabine. Cranial radiation was given to children aged > 3 years who were not receiving high dose cytarabine. Maintenance chemotherapy consisted of oral 6-mercaptopurine and methotrexate with 3 monthly intensification with vincristine, daunomycin, L-asparginase and prednisolone. Patients having NHL were treated on MCP 842 protocol [10] with addition of moderate dose methotrexate in each cycle.

CMV testing: Testing for CMV were done by pp65 antigen analysis, qualitative or quantitative RT-PCR. Results were considered positive or negative based on laboratory determined cut-off values. If CMV positivity was documented, gancyclovir was given intravenously 5 mg/kg/dose twice a day for at least 10 days, followed by oral gancyclovir / valgancyclovir for total 21 days.

Treatment of neutropenic fever: Patients having neutropenic fever had a single baseline blood culture done. Blood cultures were repeated after every 48-72 hours if defervescence was not obtained. Patients having diarhea were screened for cryptosporidium and isospora in their stools. Ceftriaxone and Amikacin were used as the initial empiric antibiotic combination. Switch to Piperacillin-Tazobactam, Carbapenems or Vancomycin were made if fever persisted or guided by culture reports. Amphoterocin-B was used as empiric antifungal therapy if fever persisted for more than 5-7 days.

Statistical analysis: CMV positive group was compared with CMV negative group using chi-square or Fisher's exact rest for categorical variables. Duration of neutropenia was compared using Mann-Whitney U-test. $P < 0.05$ was considered as statistically significant.

RESULTS

We identified 24 episodes of prolonged cytopenias, based on our definition (ALL-23, NHL-1). Of these 24 episodes, CMV infection was diagnosed by pp65 antigen analysis in 6 episodes, qualitative PCR in 10 and quantitative PCR in 8 episodes. CMV testing had been done after a median duration of 13 days of cytopenia (range 4-30 days). In 2 episodes, tests were done earlier than 10 days–one child had chronic diarrhea for 2 weeks prior to cytopenia, while the other child presented with respiratory distress along with cytopenia. CMV infection was detected in 13 (54%) out of the 24 episodes of prolonged cytopenia. Eight out of these 13 episodes occurred during or immediately after prolonged courses of steroids or moderate-dose methotrexate, while the rest occurred during maintenance treatment. Neutropenia and thrombocytopenia were present in 11 and 13 cases, respectively.

***Table* I** shows the clinical characteristics of the CMV-positive cytopenia episodes. Fever was present in 11 episodes (duration 2-20 days), which started at onset of cytopenia in 5 episodes. ***Web Table* I** shows details of blood culture results, other infections and antimicrobials used in the CMV positive episodes. Watery diarrhea with mucus was present in 9 out of 13 CMV positive episodes (duration 3-60 days). Lower respiratory tract infection (fever, tachypnea, bilateral diffuse infiltrates on *X*-ray chest) was seen in only 1 patient. In children chorioretinitis was seen about 3 months after the cytopenia episode. In both these episodes, decreased vision was the presenting complaint. Six out of the 13 episodes were associated with other co-infections (bacterial-3, Candidemia-1, *Cryptosporidium parvum* diarrhea-1, Varicella-1).

Gancyclovir was used for all CMV positive episodes, except one, where spontaneous recovery of counts was noted prior to availability of CMV report. In treated episodes, counts recovered sufficiently for further chemotherapy to be restarted in all but 1 episode after a median duration of 8 days (range 3-56 days). In 7/12 episodes (spontaneous recovery was seen in 1 episode before gancyclovir), platelet counts improved by >30,000/ mm^3 above baseline counts without transfusion support within 3 days of starting gancyclovir. In one episode, cytopenia failed to resolve after 25 days of gancyclovir therapy and the patient died of neutropenic fever in the same episode. No side effects attributable to gancyclovir were noted. None of the episodes where response to gancyclovir occurred showed any further deterioration in hematological parameters while on gancyclovir therapy.

***Table* II** shows comparison between CMV positive and negative episodes. Duration of cytopenia was not significantly different between the two groups. In the CMV positive group, in addition to the mortality during cytopenia episode described above, 2 more patients (patient 5 and 8 in ***Table* I**) died subsequently, outside the study period, as a result of CMV infection. First patient developed CMV colitis, which did not respond to gancyclovir therapy. Cidofovir could not be used due to financial constraints. The second patient developed recurrence of chorioretinitis and recurrent cytopenias towards the end of the maintenance chemotherapy. CMV

TABLE I CLINICAL CHARACTERISTICS OF CYTOPENIA EPISODES DUE TO CMV INFECTIONS

Pt no	*Platelet nadir	*ANC nadir	Duration of cytopenia (d)	Fever	LM	Fundoscopy	Recovery after GCV (d) Symptom	Cytopenia	Remarks
1	17	0.4	28	Yes	No	Chorioretinitis, bilateral	Prior	Prior	Developed chorio-retinitis 3 mo later
2	33	0.15	26	Yes	Yes	ND	Prior	3	—
3	31	0.03	42	Yes	No	Normal	7	21	Altered sensorium, CSF and MRI normal
4	5	2.5	21	Yes	No	–	5	12	Severe pain abdomen
5a	10	0.2	126	Yes	Yes	–	5	56	—
5b	12	0	58	Yes	Yes	Normal	3	6	—
5c	68	1	78	No	Yes	–	14	?	—
6a	3	0	50	Yes	Yes	–	28	17	—
6b	3	0.06	16	No	Yes	–	4	8	—
6c	5	0.02	52	Yes	Yes	–	no	No	Persistent vomiting 1.5 mo prior to onset of cytopenia; died in same episode
7	51	0.3	14	Yes	Yes	–	5	7	—
8	14	0.5	14	Yes	No	Normal	7	6	Developed chorio-retinitis 3 mo later
9	16	0.1	18	Yes	Yes	Chorioretinitis, unilateral	2	6	—

*ANC: absolute neutrophil count; GCV: gancyclovir; LM: loose motions; *$\times 10^9$/L.*

TABLE II COMPARISON BETWEEN CMV POSITIVE AND NEGATIVE EPISODES OF CYTOPENIA

Criteria	*CMV positive*	*CMV negative*
Number	13	11
Cytopenia duration[$]	28 d (18-52)	31 d (22-35.5)
Fever	11	7
Positive blood culture	3/8	0/5
Loose motions[#]	9	3
Other infections	4	0
Mortality[†]	1*	0

*[†]during cytopenia episode; *2 other patients subsequently died as a result of CMV infection outside the study period; [#]P<0.05; [$]Median (IQR).*

PCR showed 3,72,000 copies/mL. Bone marrow was in remission. Parents refused further treatment and she died 3 weeks later. There has been no non-relapse mortality in the CMV negative group to date.

DISCUSSION

Our study shows that a large proportion of patients having prolonged cytopenia after chemotherapy for ALL or NHL, which was not explainable on the basis of chemotherapy toxicity, had evidence of CMV infection. CMV related morbidity and mortality has been well described in the HSCT setting. Delayed engraftment, individual cytopenias, retinitis, pneumonitis and colitis have all been described [1-5,11].

Detection of CMV antigen or DNA in blood is an indicator of CMV infection. To diagnose CMV disease, symptoms consistent with CMV disease together with detection of CMV in an appropriate specimen from the involved tissue is necessary (*e.g.* Broncho-alveolar lavage for CMV pneumonitis, CMV inclusions on colonic biopsy, etc.) [1,2,12-14]. In the allogeneic transplant setting, weekly screening for CMV is recommended for the first 100 days (longer if GVHD present or prior CMV reactivation documented) [1,3]. There is a strong co-relation between detection of CMV viremia and subsequent development of CMV disease in allogeneic HSCT patients [15,16]. Hence, pre-emptive treatment with gancyclovir is recommended if antigenemia or PCR is positive, even in the absence of overt symptoms related to CMV disease [1,3,17]. There are no universally accepted cutoffs for PCR positivity, which vary between centers and may range from >100 copies/mL to > 1000 copies/mL based on the clinical situation and treatment

center [17]. In contrast to the data and guidelines available regarding CMV prophylaxis, screening and treatment for allogeneic HSCT patients, there are very few reports regarding the incidence, risk factors and outcome of CMV infection in patients having ALL or NHL receiving conventional chemotherapy [6-9]. Our study shows that CMV infection was present in a large proportion of patients having prolonged 'unexplained' cytopenia after chemotherapy for ALL or NHL. Other manifestations of CMV disease such as colitis, chorioretinitis and pneumonitis were also simultaneously or sequentially present in many patients.

The prompt recovery of cytopenias in a majority of our patients after starting gancyclovir also supports the causative role played by CMV infection. Although our patients benefited due to gancyclovir therapy, our study is not designed to address the issue of treatment for CMV in this patient population.

Unlike in the developed nations, where CMV seropositivity is seen in 30-40% of the population, the incidence of CMV seropositivity in India is very high, reaching upto 95% by about 5 years of age [18]. CMV infection, once acquired, remains latent in the leukocytes. Reactivation would occur in cases of severe and prolonged suppression of T-cell immunity. Myelosuppression due to CMV infection occurs either by direct infection of progenitor cells or by infection of stromal cells leading to decreased growth factor production [19,20]. Most of the prolonged cytopenia episodes in our series have occurred following prolonged courses of steroids or moderate-dose methotrexate. Both these agents have significant immunosuppressive activity. With use of more aggressive "western" protocols for treatment of ALL in Indian children, increase in the incidence of CMV re-activation and disease is likely to occur.

Fever was present in a majority, either due to CMV infection or to secondary bacterial or fungal infection. CMV infection in the HSCT setting has been associated with increased mortality due to bacterial or fungal pathogens [21-22]. In our study, 46% of CMV positive patients had evidence of other co-existing pathogens. The increased non-relapse mortality in the CMV positive group as compared to the CMV negative group indicates the seriousness of the problem even in patients receiving conventional chemotherapy. Increased risk of other pathogens along with CMV reactivation may be due to an overall immunosuppression contributing to both CMV reactivation as well as other opportunistic bacterial or fungal pathogens, or due to an immunomodulatory effect of CMV infection itself [21-22].

Two out of the three patients who had chorioretinitis developed it few months after other manifestations had been successfully treated. No other evidence of CMV infection was present in these patients at the time of detection of chorioretinitis. Similar late occurrence of CMV chorioretinitis has been described in the HSCT setting [1, 11]. Our study is likely to underestimate the incidence of CMV chorioretinitis. Milder cases with spontaneous resolution may have been undetected. Also, fundoscopy data was not available in a majority of our cases. Patients having non-ocular CMV disease should have periodic screening for next few months for detection of chorioretinitis.

In conclusion, our study shows that CMV infection can cause prolonged cytopenias even after conventional chemotherapy for ALL or NHL. Serious morbidity or treatment delays can occur as a result. This finding is important in the Indian context, where CMV sero-positivity is almost universal among children. With increasing use of more aggressive chemotherapeutic regimens for treatment of childhood ALL or NHL in India, we are likely to see a higher incidence of CMV reactivation. In the absence of clear-cut screening guidelines in the non-HSCT setting, early suspicion of CMV disease is necessary. Our study suggests that cytopenia that is prolonged or not explainable on the basis of chemotherapy toxicity should be evaluated for CMV infection, so that antiviral treatment can be instituted early and chemotherapy delays avoided.

Acknowledgments: Dr Atul Mulay and KRNST for their valuable inputs regarding data analysis and manuscript preparation.
Contributors: SK: study design, data collection, analysis and interpretation, drafting the manuscript; PB: data analysis and interpretation, drafting the manuscript; SP: data analysis and interpretation, drafting the manuscript. All authors approved the final manuscript. SK will be the guarantor for this study.
Funding: None; *Competing interests*: None stated.

REFERENCES

1. Ljungman P, Reusser P, de la Camara R, Einsele H, Engelhard D, Ribaudi P, *et al*. Management of CMV infections: recommendations from the infectious diseases working party of the EBMT. Bone Marrow Transplant. 2004;33:1075-81.
2. Zaia J. Prevention and management of CMV-related problems after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2002;29:633-8.
3. Fraser G, Walker I, and the Canadian Blood and Marrow Transplant Group. Cytomegalovirus prophylaxis and treatment after hematopoietic stem cell transplantation in Canada: A description of current practices and comparison with Centers for Disease Control / Infectious Diseases Society of America / American Society for Blood and Marrow Transplantation guideline recommendations. Biol

WHAT IS ALREADY KNOWN?

- CMV infection produces cytopenia after the very intense immunosuppression associated with hematopoietic stem cell transplantation.

WHAT THIS STUDY ADDS?

- CMV infection can cause similar cytopenia even after standard dose chemotherapy for ALL / NHL. Cytopenia which is prolonged or unexplained on the basis of chemotherapy toxicity should be evaluated for CMV infection.

Blood Marrow Transplant. 2004;10:287-97.
4. Montesinos P, Sanz J, Cantero S, Lorenzo I, Martin G, Saavedra S, *et al*. Incidence, risk factors, and outcome of cytomegalovirus infection and disease in patients receiving prophylaxis with oral valganciclovir or intravenous ganciclovir after umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2009;15:730-40.
5. Dominietto A, Raiola A, van Lint M, Lamparelli T, Gualandi F, Berisso G, *et al*. Factors influencing haematological recovery after allogeneic haemopoietic stem cell transplants: graft-versus-host disease, donor type, cytomegalovirus infections and cell dose. Br J Haematol. 2001;112:219-27.
6. Castagnola E, Cristina E, Dufour C. High-dose oral gancyclovir for management of CMV-symptomatic infection in a child with acute lymphoblastic leukemia. Med Pediatr Oncol. 2002;38:295-6.
7. Armstrong D, Haghbin M, Balakrishnan S, Murphy M. Asymptomatic cytomegalovirus infection in children with leukemia. Am J Dis Child. 1971;122:404-7.
8. Nguyen Q, Estey E, Raad I, Rolston K, Kantarjian H, Jacobson K, *et al*. Cytomegalovirus pneumonia in adults with leukemia: An emerging problem. Clin Infect Dis. 2001;32:539-45.
9. Chemaly R, Torres H, Hachem R, Nogueras G, Aguilera E, Younes A, *et al*. Cytomegalovirus pneumonia in patients with lymphoma. Cancer. 2005;104:1213-20.
10. Advani S, Pai S, Adde M, Vaidya S, Vats S, Naresh K, *et al*. Preliminary report of an intensified, short duration chemotherapy protocol for the treatment of pediatric non-Hodgkin's lymphoma in India. Ann Oncol. 1997;8:893-7.
11. Crippa F, Corey L, Chuang EL, Sale G, Boeckh M. Virological, clinical, and ophthalmologic features of cytomegalovirus retinitis after hematopoietic stem cell transplantation. Clin Infect Dis. 2001;32:214-9.
12. Boeckh M, Bowden RA, Goodrich JM, Pettinger M, Meyers JD. Cytomegalovirus antigen detection in peripheral blood leukocytes after allogeneic marrow transplantation. Blood. 1992;80:1358-64.
13. 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.
14. Avery RK, Adal KA, Longworth DL, Bolwell BJ. A survey of allogeneic bone marrow transplant programs in the United States regarding cytomegalovirus prophylaxis and pre-emptive therapy. Bone Marrow Transplant. 2000;26:763-7.
15. 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.
16. 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.
17. Boeckh M, Ljungman P. How I treat cytomegalovirus in hematopoietic cell transplant recipients. Blood. 2009;113:5711-9.
18. Venkitaraman A, Seigneurin J, Lenoir G, Jacob John T. Infections due to the Human Herpesviruses in Southern India: A Seroepidemiological Survey. Int J Epidemiol. 1986;15:561-6.
19. Sing G, Ruscetti F. Preferential suppression of myelopoiesis in normal human bone marrow cells after in vitro challenge with human cytomegalovirus. Blood. 1990;75:1965-73.
20. Simmons P, Kaushansky K, Torok-Storb B. Mechanisms of cytomegalovirus-mediated myelosuppression: Perturbation of stromal cell function versus direct infection of myeloid cells. Proc Natl Acad Sci USA. 1990;87:1386-90.
21. Nichols W, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)–seronegative recipients of stem cell transplants from seropositive donors: Evidence for indirect effects of primary CMV infection. J Infect Dis. 2002;185:273-82.
22. Humar A, Wood S, Lipton J, Messner H, Meharchand J, McGeer A, *et al*. Effect of cytomegalovirus infection on 1-year mortality rates among recipients of allogeneic bone marrow transplants. Clin Infect Dis. 1998;26:606-10.