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Monitoring of cytomegalovirus infection in non-transplant pediatric acute lymphoblastic leukemia patients during chemotherapy

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Abstract

Cytomegalovirus (CMV) infection is a significant cause of morbidity and mortality in the posttransplant setting; however, it is increasingly recognized in pediatric leukemia during chemotherapy. This study assessed the prevalence and associated factors of CMV infection in pediatric non-transplant leukemia patients.

This was a cross-sectional study of 50 pediatric acute lymphoblastic leukemia (ALL) patients receiving chemotherapy at Ramathibodi Hospital from December 2015 to December 2016. CMV viral load quantified by DNA polymerase chain reaction (PCR) was monitored in different phases of chemotherapy: enrolment, post-induction, post-consolidation, post-intensification, and maintenance.

One hundred forty one blood tests were evaluated from 50 patients. Overall prevalence of CMV DNAemia ($\geq$20 copies/mL) and high-level CMV DNAemia ($\geq$1000 copies/mL) was 52% (26 of 50) and 16.0% (8 of 50), respectively. All patients with high-level CMV DNAemia were in the maintenance phase of chemotherapy. One patient had CMV retinitis, while the rest had no end-organ CMV diseases. Increased lymphocyte count was significantly associated with protection from high-level CMV DNAemia (odds ratio 0.997, $P = .02$). Receiver operating characteristic curve identified a cut-off value of 798 cells/mm^3 of absolute lymphocyte count (ALC) as a discriminator for the presence of high-level CMV DNAemia (area under the curve 0.756, 95% CI 0.645–0.867, $P = .001$) with 88.9% sensitivity and 50.4% specificity.

CMV infection predominantly occurred during maintenance chemotherapy. Low ALC was significantly associated with high-level CMV DNAemia. CMV infection surveillance by quantitative CMV DNA PCR during maintenance chemotherapy in patients with ALC <800 cells/mm^3 may be considered.

Abbreviations: ALC = absolute lymphocyte count, ALL = acute lymphoblastic leukemia, ALP = alkaline phosphatase, ALT = alanine aminotransferase, ANC = absolute neutrophil count, AST = aspartate aminotransferase, CMV = cytomegalovirus, GGT = gamma glutamyl transferase, HSCT = hematopoietic stem cell transplantation, IQR = interquartile range, PCR = polymerase chain reaction, ROC = receiver operating characteristic, WBC = white blood count.

Keywords: acute lymphoblastic leukaemia, CMV infection, pediatric

1. Introduction

Cytomegalovirus (CMV) is prevalent worldwide and is usually acquired during childhood. The seroprevalence of CMV infection in Thai children is around 70%.[1] As with all herpesviruses, CMV establishes lifelong latency in the host after primary infection. Activation from this latent state can occur after immunosuppression.[2] While the humoral and innate immune responses play a role in the early phase of infection, cellular immunity is required to control its latency, prevent reactivation, and inhibit progression to disease.[3] Disease severity varies in different hosts, from mild disease in healthy individuals, to infectious mononucleosis syndrome in young adults, and severe end-organ diseases in those with defective T-cell-mediated immunity, such as human immunodeficiency virus infection, hematopoietic stem cell transplantation, and solid organ transplant recipients.

Standard chemotherapy for pediatric leukemia causes depletion of humoral immunity while cellular immunity is less affected.[4] CMV reactivation during chemotherapy for lymphoblastic leukemia is rare and insignificant; nevertheless, there have been increasing reports regarding CMV end-organ diseases, especially CMV retinitis in this setting.[5–13] We postulate that CMV reactivation occurs at some points during chemotherapy. Without any intervention, this may finally progress to end-organ

diseases.[14] However, data regarding the true impact and time course of CMV infection in non-transplant pediatric leukemia during chemotherapy are limited. Our primary objective was to assess the prevalence of CMV infection by active monitoring of quantitative CMV DNA polymerase chain reaction (PCR) in different phases of chemotherapy. Our secondary objective was to identify factors associated with CMV infection in pediatric leukemia patients.

2. Materials and methods

2.1. Study design and population

This was a cross-sectional, single-center study of 50 pediatric acute lymphoblastic leukemia patients aged <18 years conducted at the Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Thailand from December 2015 to December 2016. All patients had been treated with standard chemotherapy using either RAMAALL protocol, our institutional protocol, or ThaiPOG, a Thai Pediatric Oncology Group protocol. None of the patients received hematopoietic stem cell transplantation. The study was approved by the Ethics Committee of Ramathibodi Hospital. Upon being informed about the study protocol, no eligible patients refused to be enrolled in the study. After receiving parental written informed consent for study participation, the patients were consecutively enrolled. CMV serology was tested at the time of enrolment. CMV viral load was monitored according to their phases of chemotherapy at enrolment, post-induction, post-consolidation, post-intensification, and then every 3 months during the maintenance phase until the end of treatment. The process of enrollment is demonstrated in Figure 1.

2.2. Detection and definition of CMV infection

Quantitative CMV DNA PCR from blood samples was performed by the Abbott RealTime CMV assay (Abbott Molecular Inc., Des Plaines, IL). Sample preparation was carried out on m2000sp using the magnetic bead m2000 System DNA extraction kit, and amplification and detection of the UL34 and UL80.5 genes of CMV were conducted on the m2000rt using RealTime CMV kits. The quantification linear range of plasma was from 20 to 100,000,000 copies/mL (31–156,000,000 IU/mL; 1 copy/mL being equivalent to 1.56 IU/mL for this kit). CMV infection is defined as detection of viral nucleic acid in any body fluid or tissue specimens. CMV DNAemia is defined as the detection of CMV DNA in samples of plasma, serum, whole blood, or isolated peripheral blood lymphocytes, or in buffy coat specimens.[14] In this study, we defined high-level CMV DNAemia as having quantitative CMV DNA in plasma >1000 copies/mL (1561 IU/mL).

2.3. Statistical analysis

STATA version 14.2 was used to perform all statistical analyses. Quantitative variables were described by median and interquartile range (IQR) and qualitative variables were described by frequency (percentage). Laboratory data were compared between patients with high-level CMV DNAemia (≥1000 copies/mL) and low-level DNAemia (<1000 copies/mL or) by median regression analysis. Since there were multiple measures per individual for some individuals, the associated factors for high-level CMV DNAemia were analyzed by mixed logistic regression analysis to account for the potential intraindividual clustering. The levels of CMV DNAemia after treatment with ganciclovir or Valganciclovir were not included in the analysis. The variables that may be associated with high-level CMV DNAemia include total white blood count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), hemoglobin, platelet count, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), total protein, albumin, and total bilirubin. $P < .05$ was considered to indicate a statistically significant difference.

3. Results

Fifty acute lymphoblastic leukemia patients (30 boys and 20 girls) were enrolled. The median age of the patients was 7 years (IQR 4–11). CMV serology was obtained from 48 patients, and
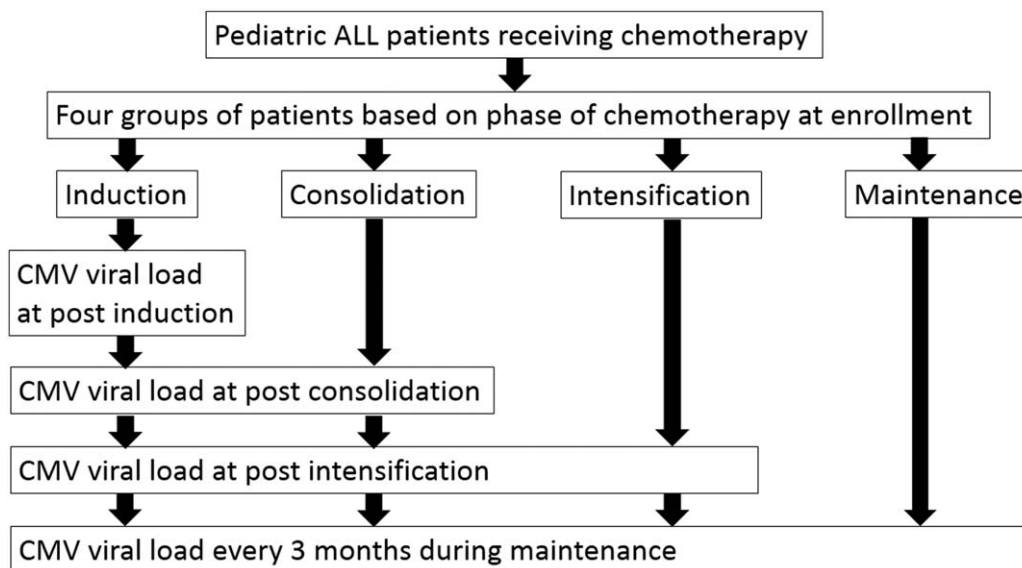

Figure 1. Process of enrollment. Pediatric ALL patients were enrolled in the study at different phases of chemotherapy. The time for monitoring CMV DNA quantitation was according to the phase of chemotherapy at enrollment. ALL=acute lymphoblastic leukemia, CMV=cytomegalovirus.

Table 1

Demographic data of the enrolled ALL patients.

Characteristics	Number of patients (50)
Male	30 (60.0%)
Age in years, median (IQR)	7 (4–11)
Positive CMV serology at baseline (n = 48)	37 (77.1%)
Phase of chemotherapy at enrollment	
Post-induction	8 (16.0%)
Post-consolidation	5 (10.0%)
Post-intensification	2 (4.0%)
Maintenance	35 (70.0%)

ALL = acute lymphoblastic leukemia, CMV = cytomegalovirus, IQR = interquartile range.

Table 2

Laboratory tests of patients with high-level CMV DNAemia (≥1000 copies/mL) and low-level CMV DNAemia (<1000 copies/mL).

Laboratory parameters	High-level CMV DNAemia Median (IQR)	Low-level CMV DNAemia Median (IQR)	P
WBC (cells/μL)	2760 (2090–3435)	2950 (2140–4620)	.789
ANC (cells/μL)	1954 (1008–2348)	1782 (1301–3053)	.649
ALC (cells/μL)	360 (198–785)	793 (461–1230)	.008*
Hemoglobin (g/dL)	11.2 (10.5–11.5)	11.5 (10.3–12.2)	.507
Platelet count (×10³ cells/μL)	262 (152.5–310.5)	219 (161–305)	.605
AST (U/L)	51 (44.0–81.5)	36 (28–49)	.002*
ALT (U/L)	99 (81.5–196.5)	54 (25–124)	.020*
ALP (U/L)	143 (106–187.5)	199 (161–238)	.004*
GGT (U/L)	20 (13–43)	20 (13–35)	1.000
Total protein (g/L)	58.8 (57.7–60.5)	61.1 (58.1–64.4)	.085
Albumin (g/L)	38.6 (37.7–40.7)	38.4 (36–40.4)	.902
Total bilirubin (mg/dL)	0.6 (0.5–0.8)	0.5 (0.4–0.7)	.250

ALC = absolute lymphocyte count, ALP = alkaline phosphatase, ALT = alanine aminotransferase, ANC = absolute neutrophil count, AST = aspartate aminotransferase, CMV = cytomegalovirus, GGT = γ-glutamyl transferase, WBC = total white blood cell count.
* $P < .05$.

38 (77.1%) had positive CMV IgG at baseline. Table 1 summarizes the demographic characteristics of the study population. We evaluated 141 blood tests from 50 patients: 8, 9, 8, 38, and 78 tests were classified as post-induction, post-consolidation, and post-intensification, <1 year of maintenance, and >1 year of maintenance, respectively.

The overall prevalence of CMV DNAemia (lower limit of detection of CMV DNA at 20 copies/mL) was 26 of 50 (52%) in the pediatric acute lymphoblastic leukemia patients. All were positive for CMV IgG. None of the patients who were negative for CMV IgG developed CMV DNAemia. Classified by phase of chemotherapy, the prevalence of CMV DNAemia was 4 of 8 (50%), 4 of 9 (44.4%), 1 of 8 (12.5%), 6 of 19 (31.6%), and 14 of 26 (53.9%) at post-induction, post-consolidation, and post-intensification, <1 year of maintenance, and >1 year of maintenance, respectively.

The overall prevalence of high-level CMV DNAemia (≥1000 copies/mL) was 8 of 50 (16%). All patients with high-level CMV DNAemia were in the maintenance phase of chemotherapy. One of eight patients was in the first year of maintenance, while the others were in maintenance phase for >1 year. Figure 2 demonstrates the prevalence of CMV DNAemia and high-level CMV DNAemia. Only one patient in this study died. He had had high-level CMV DNAemia, and had been treated with ganciclo-

vir for 3 weeks at which time the CMV viral load was suppressed. He presented 2 weeks later with septic shock and succumbed to this.

Table 2 shows the results of laboratory tests in patients with high-level CMV DNAemia (≥1000 copies/mL) episodes and low-level CMV DNAemia (<1000 copies/mL) episodes. Absolute lymphocyte count (ALC) and alkaline phosphatase (ALP) were significantly lower but aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were higher in high-level CMV DNAemia episodes when compared with low-level CMV DNAemia episodes.

When comparing the laboratory parameters between high-level and low-level CMV DNAemia episodes (Table 3), we found that more profound lymphopenia was observed among high-level CMV DNAemia episodes (median 360, IQR 198–785) when
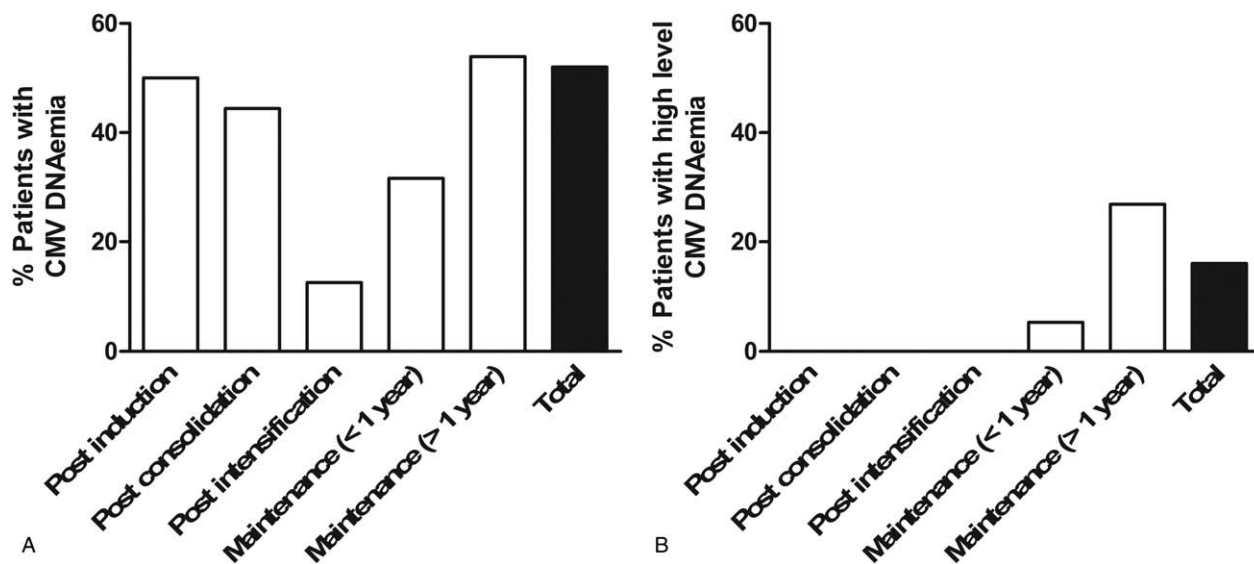

Figure 2. Prevalence of CMV DNAemia. Prevalence of CMV DNAemia (A) and of high-level CMV DNAemia (B) at different phases of chemotherapy is demonstrated. The numbers above the bars represent the number of patients with CMV DNAemia/the number of patients in that group. CMV = cytomegalovirus.

Table 3

Factors associated with high-level CMV DNAemia in pediatric lymphoblastic leukemia patients.

Variables	High-level CMV DNAemia	Low-level CMV DNAemia	OR (95%CI)	P
ALC (cells/μL)	360 (198–785)	793 (461–1230)	0.997 (0.994–0.999)	.020[*]
AST (U/L)	51 (44.0–81.5)	36 (28–49)	1.004 (0.979–1.031)	.741
ALT (U/L)	99 (81.5–196.5)	54 (25–124)	1.000 (0.992–1.008)	.955
ALP (U/L)	143 (106–187.5)	199 (161–238)	0.984 (0.967–1.001)	.072

ALC = absolute lymphocyte count, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CMV = cytomegalovirus.
[*] $P < .05$.

compared with low-level CMV DNAemia episodes (median 793, IQR 461–1230). ALC was shown to be the only independent factor significantly associated with high-level CMV DNAemia (OR 0.997, 95% CI 0.994–0.999, $P = .02$) by mixed logistic regression model. For every 1 lymphocyte/μL increase, the chance of having high-level CMV DNAemia decreased by 0.3% (OR 0.997, 95% CI 0.994–0.999, $P = .02$). Therefore, an increase of 100 lymphocytes would decrease the chance of having high-level CMV DNAemia by 26%. Eighty-eight percent (7 of 8) of patients with high-level CMV DNAemia had lymphopenia according to the definition of ALC <1000/μL, while the other patient had ALC of 1200/μL.

Figure 3 shows the relationship between blood CMV viral load and ALC in two patients. A decrease in CMV viral load was associated with an increase in ALC.

In our study protocol, treatment for CMV infection was at the discretion of the attending physicians. Among eight patients with high-level CMV DNAemia, five received treatment with intravenous ganciclovir with or without subsequent oral valganciclovir. Duration of antiviral treatment ranged from 1 to 5 weeks depending on the response of the follow-up quantitative CMV DNA PCR results. Seven patients were asymptomatic and had no CMV end-organ diseases at the time of diagnosis, except for Patient 8, who presented with febrile neutropenia and shock and was first diagnosed with sepsis. Her blood cultures were negative for bacteria but quantitative PCR for CMV was positive at 1,307,730 copies/mL. Even though her vision was normal, ophthalmological examination revealed bilateral CMV retinitis. Her symptoms improved after treatment with ganciclovir. All patients responded well to treatment and no drug resistance was noted. Patient five had recurrent infection at 6 months when he presented with fever and diarrhea. Among three patients who did not receive ganciclovir treatment, two had

viral suppression at 1 and 4 months, respectively. The other patient still had CMV DNAemia of 723 copies/mL without any symptoms at the end of our study. Table 4 summarizes the details of the eight pediatric acute lymphoblastic leukemia patients with high-level CMV DNAemia.

We next attempted to discriminate the cut-off point of ALC that could be used to discriminate between high-level and low-level CMV DNAemia episodes in patients with CMV DNAemia. The receiver operating characteristic (ROC) curve revealed that ALC <798/μL discriminated high-level from low-level CMV DNAemia, with an area under the curve of 0.756 (95% CI 0.645–0.867; $P < .001$), a sensitivity of 88.9% and specificity of 50.4% (Fig. 4).

4. Discussion

This single center, cross-sectional study is believed to be the first study to perform active monitoring of CMV in children with asymptomatic non-transplant acute lymphoblastic leukemia (ALL). Our study revealed that CMV infection was common among these patients as the overall prevalence of CMV DNAemia was 52%, indicating that half of pediatric ALL patients developed CMV reactivation during chemotherapy. However, most CMV infections in these patients were asymptomatic.

The cut-off level of quantitative CMV DNA in blood for evaluating CMV end-organ diseases and considering preemptive treatment of CMV infection among pediatric allogeneic hematopoietic stem cell transplantation (HSCT) patients varies from 100 to 1000 copies/mL, depending on the clinical situation.[15] This positive threshold was consistent with a survey conducted among European bone marrow transplant centers to evaluate CMV monitoring and treatment of pediatric HSCT patients.[16] However, data regarding treatment strategy and outcomes of
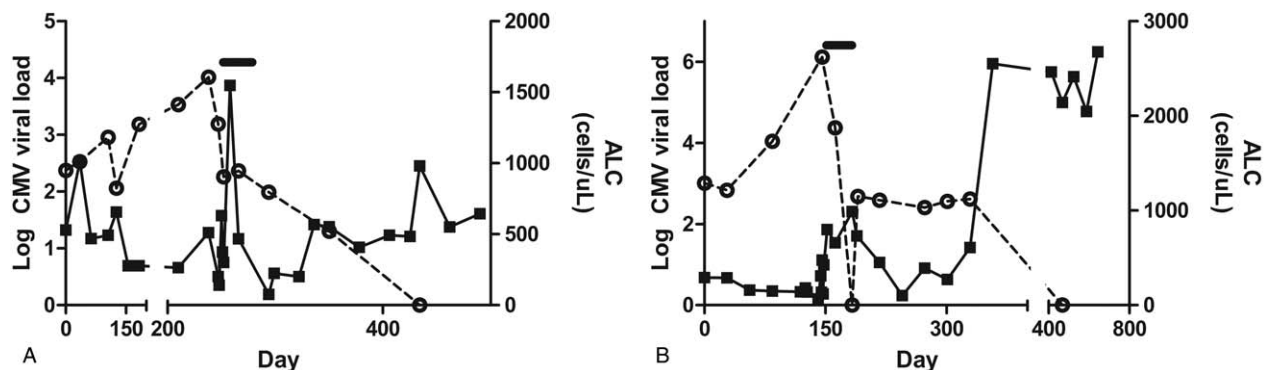

Figure 3. Association between level of CMV DNA and ALC. Examples of two patients who had CMV DNAemia are shown. Day 0 represents when the first CMV DNAemia was detected. Black line (—) represents the period when the patients received ganciclovir/valganciclovir; ■ represents ALC; ● represents CMV DNA level. CMV = cytomegalovirus.

Table 4

Patients with high CMV DNAemia.

No	Age (y)	Sex	Phase of chemotherapy	Max. CMV viral load (copies/mL)	ANC <1500/μL	ALC < 1500/μL	Hepatitis	Retinitis	Colitis	Pneumonitis	Treatment	Outcome
1	11	F	Maintenance <1 y	10,818	Yes	Yes	No	No	No	No	No	Virus suppressed in 4 months
2	6	M	Maintenance ≥1 y	14,220	Yes	Yes	Yes	No	No	No	Ganciclovir/ valganciclovir 5 weeks	Virus suppressed
3	4	M	Maintenance ≥1 y	10,388	No	Yes	No	No	No	No	ganciclovir 1 week	Virus suppressed
4	13	F	Maintenance ≥1 y	4969	No	No	Yes	No	No	No	No	Virus suppressed in 1 month
5	16	M	Maintenance ≥1 y	18,424	No	Yes	Yes	No	No	No	Ganciclovir 2 weeks	Virus suppressed but recurrence in 6 months
6	4	F	Maintenance ≥1 y	1994	No	Yes	No	No	No	No	No	Viral load 723 copies/mL at the end of the study
7	6	M	Maintenance ≥1 y	13,495	No	Yes	Yes	No	No	No	Ganciclovir 3 weeks	Virus suppressed but expired 2 weeks later because of septicemia
8	16	F	Maintenance ≥1 y	1,307,730	No	Yes	Yes	Yes	No	No	Ganciclovir 3 weeks	Virus suppressed

CMV infection in non-transplant settings are still lacking, thus, we consequently used a cut-off level of 1000 copies/mL to define high-level CMV DNAemia. All high-level CMV DNAemia patients
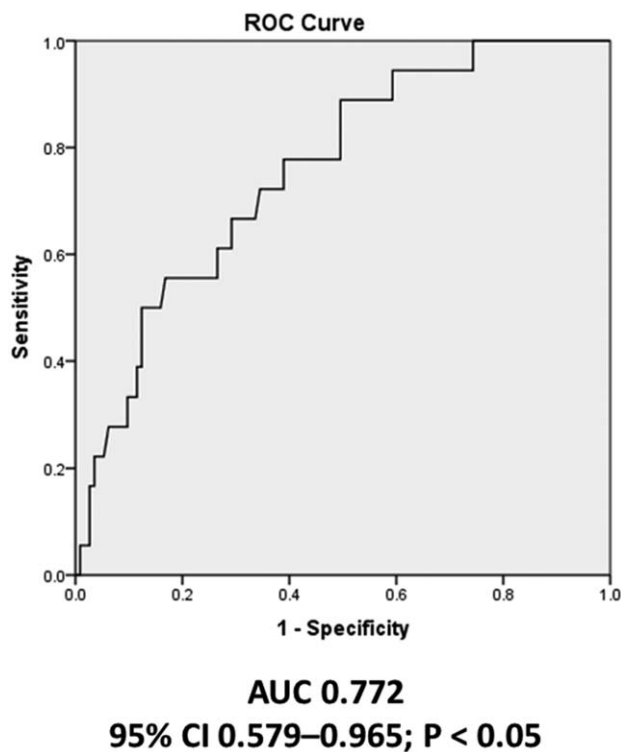

Figure 4. ROC curve of ALC in discriminating between the presence or absence of high-level CMV DNAemia. AUC of the ROC curve was 0.756 (0.645–0.867, P=.001). ALC=absolute lymphocyte count, CMV=cytomegalovirus.

were in maintenance phase of chemotherapy. These patients had positive CMV serology at baseline, implying previous CMV exposure. CMV DNAemia was, therefore, likely to have resulted from reactivation of latent virus rather than primary infection during chemotherapy. This result was consistent with previous studies regarding maintenance phase as the most common period of CMV infection in pediatric lymphoblastic leukemia.[6,10] Despite more intense chemotherapy in the prior phases, CMV reactivation tended to occur during maintenance phase. The pathogenesis of CMV reactivation during maintenance phase remains unclear. Cell-mediated immunity is essential in counteracting CMV infection. Immune function during chemotherapy for lymphoblastic leukemia is commonly marked by B-cell depletion, while T-cells and natural killer cells are less affected.[4] However, a study of immune reconstitution during maintenance therapy reported that lymphopenia was commonly observed and T-cell reconstitution was delayed during the maintenance phase.[17]

Our finding supports the hypothesis that depletion of lymphocytes during maintenance phase may play an important role in CMV reactivation. Corticosteroids are the main drugs for treatment of ALL during the maintenance phase. Lymphopenia caused by these drugs may contribute to more prevalent CMV reactivation during this phase rather than in the induction or consolidation phases.[18] Another possibility is that CMV reactivation may lead to lymphopenia as there has been evidence showing that CMV could infect hematopoietic cells and cause cytopenia.[19,20] This can in turn hinder subsequent CMV clearance.[21] The temporal relationship between CMV viral load and lymphocyte count clearly shows the inverse relationship between these two parameters. However, our study could not determine which parameter was the cause of the other.

It has long been known that increased CMV viral load in peripheral blood in immunocompromised individuals predisposes towards end-organ diseases.[22–24] However, end-organ diseases can even develop in low-level CMV DNAemia, as

reported in patients after solid organ transplantation.[25] The correlation between CMV viral load and end-organ disease is still not clear in ALL patients in a non-transplant setting. Our study showed that only 1 of 8 patients who had high-level CMV DNAemia developed retinitis. This patient had CMV blood viral load $>10^6$ copies/mL. It is possible that she might have had long-standing CMV viremia before presentation. Had she had CMV viremia detected earlier and been treated, she might not have had retinitis. This scenario also pointed out that ALL patients presenting with sepsis but negative bacterial culture may have underlying CMV reactivation or infection.

In our resource-limited setting, up-front screening of quantitative CMV DNA PCR in every case of pediatric leukemia and applying preemptive treatment for CMV infection can be expensive and may not be feasible. High-level CMV DNAemia was associated with lymphopenia, and the cut-off level of <800 lymphocytes/μL gave the optimal sensitivity and specificity. Therefore, ALL patients who are in maintenance phase and develop lymphopenia (ALC < 800/μL) may be considered for screening with quantitative CMV DNA PCR. Presence of CMV reactivation may require further viral load monitoring to document whether viral load is declining or rising. Rising of the viral load may need pre-emptive antiviral therapy to prevent end-organ diseases. From our study, although the prevalence of high-level CMV DNAemia was 16% (8 of 50), only one patient developed end-organ disease. Three patients who did not receive antiviral treatment remained well during the entire study and no end-organ diseases were detected. It remains unclear which cut-off level of CMV DNAemia implies clinical significance and which is a positive threshold for preemptive treatment in pediatric leukemia in a non-transplant setting. The threshold of treatment still varies among different settings and institutions; for example, in a pediatric HSCT setting, positive thresholds of 1000 and 5000 copies/mL were used in some centers,[16] whereas, in a solid organ transplant setting, a positive threshold of 2600 copies/mL was proposed for starting preemptive therapy.[26] To answer the question, a randomized controlled trial is needed to identify the best strategic management for CMV infection in pediatric leukemia in a non-transplant setting.

There were several limitations to our study. First, it was a cross-sectional study, and a true cause and effect could not be established. We could not conclude that lymphopenia put the patients at risk of CMV infection or that CMV infection itself caused lymphopenia. Second, a limited number of blood tests were obtained at post-induction, post-consolidation, and post-intensification; therefore, the magnitude of infection in these phases might have been underestimated. It would also be interesting to investigate further the number of CMV-specific T-cells in patients with ALL, as these cells have been shown to harbor a protective effect against CMV infection.[27,28] Reconstitution of these cells should indicate that host immunity is ready to fight against infection and toxic antiviral medications can be delayed or may not be necessary.

The strengths of our study were: first, it is believed to be the first study to assess the prevalence of CMV infection in pediatric leukemia by active monitoring of quantitative CMV DNA PCR in different phases of chemotherapy. Second, we were able to identify ALC as a significant associated factor for high-level CMV DNAemia in pediatric leukemia in a non-transplant setting. Pediatric ALL patients in maintenance phase who have had positive CMV IgG serology and experience ALC <800 cells/uL may warrant CMV reactivation surveillance.

5. Conclusion

In pediatric leukemia in a non-transplant setting, overall prevalence of high-level CMV DNAemia was 16%. CMV infection predominantly occurred during the maintenance phase of chemotherapy. Lower ALC was significantly associated with high-level CMV DNAemia. CMV infection surveillance by quantitative CMV DNA PCR may be considered in ALL patients who are in maintenance phase with ALC <800 cells/μL.

Author contributions

NP and NA designed the study and wrote the initial draft of the manuscript. JK did CMV viral load quantification. NP, NA, CT, and UA confirmed all findings and interpreted the data. SR did statistical analysis. All authors contributed to data collection and critically reviewed the article. All authors approved the final version of the article and are accountable for all aspects of the study; they will also ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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