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Low absolute neutrophilic and lymphocytic counts in children with CMV reactivation following HSCT

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***Abstract*---**Introduction: Cytomegalovirus (CMV) infection is one of the commonest infections after hemopoietic stem cell transplantation (HSCT) and it may lead to significant morbidity, mortality and can lead increased healthcare costs. Aim: The aim of this work was to evaluate the relation between neutrophilic and lymphocytic count recovery in children with CMV reactivation following HSCT. Method: The study included 30 patients with CMV reactivation who underwent allogenic hematopoietic stem cell transplantation for non-malignant diseases in Abo El-Reesh Al Mounira Pediatric Hospital, Cairo University. Pre- and post-transplant clinical and laboratory data were collected. Results: The 30 patients developed 69 episodes of CMV reactivation. 66.6% and 53.5% of the reactivation episodes were associated with low absolute lymphopenic and neutrophilic respectively. The early CMV reactivation episodes were associated with

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severe low absolute lymphocytic and neutrophilic counts with p value (0.003, 0.006) respectively. Conclusion: Neutrophilic and lymphocytic count recovery is an important factor associated with reactivation. Importance of close monitoring of viral load among lymphopenic and neutropenic patients for strong surveillance and early detection of CMV reactivation and proper management.

***Keywords*---**hematopoietic stem cell transplantation, CMV reactivation, lymphopenia, neutropenia.

Introduction

Hematopoietic stem cell transplantation (HSCT) is now believed to be a life-saving treatment for various hematological, immunological or metabolic disorders *(Parsons et al., 2013)*. Viral infections are now one of the main risks of morbidity and mortality post hematopoietic stem cell transplantation (HSCT) *(Ljungman et al., 2010)*. Cytomegalovirus (CMV) infection is the most common infection after allogeneic hemopoietic stem cell transplantation (HSCT) and it may lead to adverse transplant outcomes and increased healthcare costs *(Green et al., 2016)*. The clinical picture of CMV infection ranges from asymptomatic CMV viremia to fatal CMV disease with different organs affection *(Landolfo et al., 2003).* There are many risk factors post-HSCT that increase risk for CMV reactivation including acute or chronic graft versus host disease, recipient/donor CMV positive serology and immune suppression, caused by conditioning regimens, immunosuppressive medications and HLA disparity *(Dziedzic et al., 2017).* Recovery of immune function after HSCT is essential to control the CMV reactivation *(Blyth et al., 2016).* Preemptive therapy, that is based on PCR-based monitoring of CMV in blood, is playing an important role to improve the outcomes of CMV reactivation *(Cho et al., 2019).* The aim of this work was to evaluate the relation between neutrophilic and lymphocytic counts recovery in children with cmv reactivation following HSCT.

Patients and Methods

This was a cohort study that was carried out during the period from October 2019 to August 2021 and the patients' records were revised in the center over the previous 6 years. This study was performed at the pediatric hematopoietic stem cells transplantations center and clinical pathology department of Cairo University. The study included 30 patients who developed CMV reactivation after allo-HSCT. An ethical committee approval and a written consent of guardians were obtained from each patient. The patients' pre-transplant data included full history taking, full investigation including CMV serology. During the reactivation episodes CMV PCR viral load was monitored with associated absolute lymphocytic count (ALC) and absolute neutropenic count (ANC).

- Associated absolute lymphopenia and its grades that were defined as *(Morales et al., 2020):*
    - Grade 1: absolute lymphocytes count (ALC) 800- 999/ul\L, grade 2: ALC 500–799/uL, grade 3: ALC 200-499/uL and grade 4: ALC<200/uL.

- Grades III and IV are considered severe lymphopenia *(Grossman et al., 2015).*
- Associated absolute neutropenia that was classified as *(Newburger & Dale, 2013):* mild: 1000 to 1500/uL, moderate: 500 to 999/uL and severe: <500 /uL

All patients were routinely screened for CMV viremia with CMV PCR started 14 days after receiving stem cells. Screening was done biweekly until 100 days posttransplant, and subsequently on follow-ups once weekly, then less frequently till medical follow up decided on stopping immunosuppressive medication. The patients were screened with the real-time CMV PCR (Bosphore kit from Anatolia Geneworks) which is based on PCR principle. The pathogen is detected using fluorescent dyes that are incorporated into oligonucleotide probes. The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above background level and becomes distinguishable, is called the cycle threshold *(Li et al., 2003).* CMV reactivation was considered when CMV PCR was > 150 copies/mL *(Cohen et al., 2015)*. Early CMV reactivation was defined as that occurring during the first 100 days after stem cells transplant, while late CMV reactivation was defined as that occurring after day +100 *(Özdemir et al., 2007).*

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23 *(Čaplová and Švábová, 2020).*

Results

Among the 30 post allo-HSCT patients who developed CMV rectivation, the mean age at transplant was 4.6 years (range 0.3-14.6 years). Twenty of patients were males (66.60%) and ten (33.30%) were females. All the patients had non-malignant hematological and immunological disorders. Regarding the donors, the median age at donation was 7.8 years (range 2.6 – 35 years), 16 (53.33%) males and 14 (46.66%) were females. Two of the donors were fathers (6.70%), four were mothers (13.30%) and 24 were siblings (80.00%). All patients received peripheral blood stem cells, 29 patients of them received from HLA fully matched donor and only one patient was transplanted from HLA haploidentical related donor (3.33%). Regarding investigations during reactivation, associated investigations done included total leukocyte count (TLC), absolute neutrophilic count (ANC), absolute lymphocytic count (ALC), hemoglobin level, platelets (table 1).

During the CMV reactivation episodes the median of absolute neutrophilic count was 1806/uL (range 104-9180/uL) with 59.4% normal counts, 15.9% severe neutropenia, 18.8% moderate neutropenia, 18.8% mild neutropenia. The median of absolute lymphocytic count was 952/uL (range 0-7200/uL) with 33.3% normal counts, 18.8% severe lymphopenia (grade 3 &4), 33.3% grade 2 lymphopenia, 14.5% grade 1 lymphopenia. Early episodes of CMV reactivation were associated with low ANC and ALC respectively (figure 1,2). The majority of the early CMV reactivations episodes were associated with severe neutropenia <500/uL and severe lymphopenia grade III-IV (table 2) (figure 3). There is increase in the CMV reactivation frequency > 2 episodes among cases with low absolute lymphocytic count at D+30 (table 3).

Table (1): Complete blood count parameters during CMV reactivation

		No.= 69
Associated platelets /mm³	Median (IQR) Range	$183x\ 10^3$ ($85x10^3$ – $285x10^3$) $4x10^3$ – $667x10^3$
Associated Hemoglobin g/dl	Median (IQR) Range	9.86 ± 1.97 6 – 14.1
Associated total leukocytic count/uL	Median (IQR) Range	3500 (2200 – 5500) 100 – 14400
Associated absolute neutrophilic count/uL	Median (IQR) Range	1806 (1100 – 2880) 0 – 9180
Associated absolute lymphocytic count /uL	Median (IQR) Range	952 (620 – 1800) 0 – 7200

Table (2): Early and late onset of CMV reactivation related to neutrophilic and lymphocytic counts

		Onset of CMV reactivation		Test value	P- value	Sig.
		<100	>100			
		No.= 44	No.= 25			
Associated ANC	Normal	23 (52.3%)	18 (72.0%)	2.573	0.109	NS
	Mild	7 (15.9%)	6 (24.0%)	0.683	0.409	NS
	Moderate	3 (6.8%)	1 (4.0%)	0.232	0.630	NS
	Severe	11 (25.0%)	0 (0.0%)	7.435	0.006	HS
Associated ANC count	Median (IQR)	1672 (453.1 – 2755)	2200 (1392 – 3240)	-1.887‡	0.059	NS
	Range	0 – 9180	888 – 4740			
Associated ALC	Normal	11 (25.0%)	12 (48.0%)	3.795*	0.051	NS
	Grade I	4 (9.1%)	6 (24.0%)	2.860*	0.091	NS
	Grade II	16 (36.4%)	7 (28.0%)	0.502*	0.479	NS
	Grade III-IV	13 (29.5%)	0 (0.0%)	9.101*	0.003	HS
Associated ALC count	Median (IQR)	797(312 – 1343)	1395 (840 – 2808)	-3.224‡	0.001	HS
	Range	0 – 7200	594 – 5056			

P-value >0.05: Nonsignificant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
*: Chi-square test; ‡: Mann Whitney test

Table (3): The relation of CMV reactivation frequency with absolute lymphocytic count at D+30 (LC30)

		Frequency of reactivation		Test value	P-value	Sig.
		Frequency of reactivation <=2	Frequency of reactivation >2			
		No. = 39	No. = 13			
LC30	Median (IQR)	916 (726 – 1600)	690 (592 – 740)	-2.101‡	0.036	S

	Range	136 – 7200	440 – 1920			

P-value >0.05: Nonsignificant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS); lymphocytes at D+30 (LC30)
* Mann Whitney test

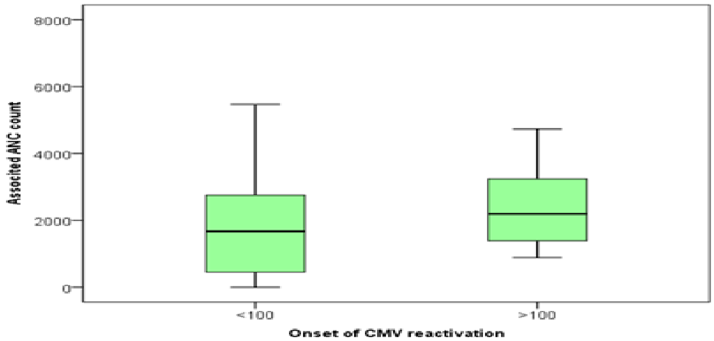

Figure (1): The absolute neutrophilic count related to the onset of CMV reactivation.

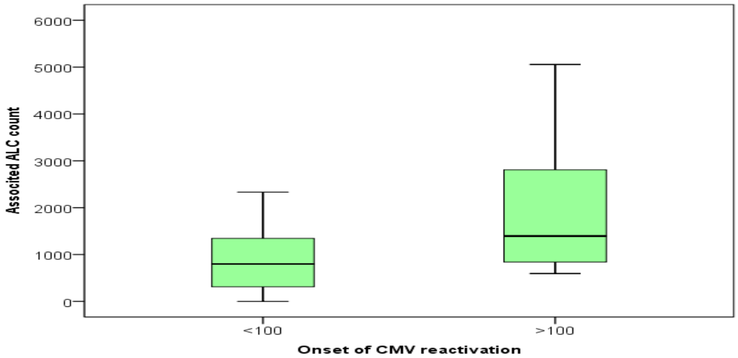

Figure (2): The absolute lymphocytic count related to the onset of CMV reactivation.

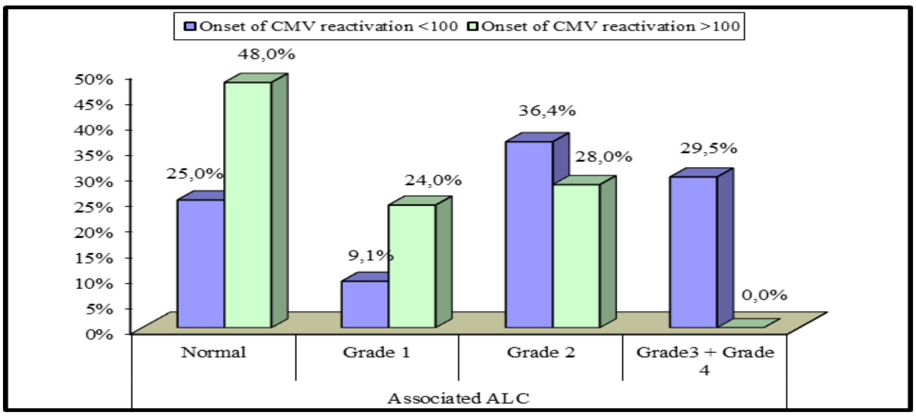

Figure (3): Delayed lymphocytic engraftment related to the onset of CMV reactivation.

Discussion

CMV reactivation is the most common viral complication after HSCT engraftment and is associated with a significant increase in mortality, which appears to be linked to post-transplant immune reconstitution *(Stern et al., 2019).* The study revealed that there was an increase in the incidence of CMV reactivation frequency in cases with low lymphocytic count at D+30, as well as a higher risk to develop early CMV reactivation before in severely lymphopenic and neutropenic patients.

Watanabe et al. (2019) observed less risk of reactivation with high lymphocytic counts and that lymphocyte count may be a good predictor for CMV reactivation. Also, *Meesing and Razonable (2018)* showed that lymphopenia is a major risk factor for CMV infection after HSCT and that a peripheral blood absolute lymphocyte count <830/µL in HSCT can serve as a marker of heigher risk for CMV infection and disease. A previous study by *Jang et al. (2012)* analyzed 43 allo-HSCT with 76 CMV reactivations. It revealed that patients with lymphopenia at D+100 post-HSCT had a higher incidence of progression to CMV disease. Also, higher rates of CMV reactivations were associated with neutropenia. *Reekie et al. (2020)* illustrated that Low lymphocytic count at the time of CMV treatment completion for the first CMV infection after HSCT was a strong predictor for CMV recurrence. The responses to treatment can be very slow in patients with severe lymphopenic counts *(Emery et al., 2013).*

Conclusion

Reactivation of CMV is common post allogenic hematopoietic stem cell transplantation in children. Delayed immune reconstruction is an important factor associated with reactivation. Importance of close monitoring of viral load among lymphopenic and neutropenic patients for strong surveillance and early detection of CMV reactivation and proper management.

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