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Post-Transplant Cyclophosphamide in Allogeneic Peripheral Blood Stem Cell Transplantation from Fully HLA- Matched Sibling Donors in Adult Egyptian Patients with Severe Aplastic Anemia

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ABSTRACT

Graft versus host disease (GvHD) is still considered a huge cause of morbidity and mortality in patients with severe aplastic anemia (SAA) who have Hematopoietic stem cell transplantation (HSCT). Objectives: our study aimed at evaluating the effectiveness of post-transplant cyclophosphamide (PTCY) as a GvHD prophylaxis in adult Egyptian patients with SAA. Materials and Methods: sixty patients with SAA who had allogeneic HSCT were divided into; Group I: 30 patients received cyclophosphamide (CY) plus cyclosporine A (CsA) as a GvHD prophylaxis regimen. Group II: 30 patients (historical controls) had received CsA and short-term methotrexate as a GvHD prophylaxis regimen (old regimen). Results: acute GvHD (aGvHD) incidence was 6.7% in group I, and 36.7% in group II. Chronic GvHD (cGvHD) occurred in 10% ,56.7% of patients in group I and II, respectively and overall survival (OS) was 70% in group I, versus 56.7% in group II. Conclusions: we concluded that PTCY / CsA showed a significant reduction in the rate of both acute and chronic GvHD with superior OS than the old regimen.

Keywords: SAA: severe aplastic anemia, HSCT: Hematopoietic stem cell transplantation, PTCY; Post-transplant cyclophosphamide, GvHD: Graft versus host disease.

1. Introduction

SAA is a rare life-threatening disease, characterized by pancytopenia and a hypocellular bone marrow (Brodsky and Jones, 2005).

A young adult patient having SAA, is best treated with allogenic hematopoietic stem cell transplantation (allo-HSCT), using human leukocyte antigen (HLA) matched related donor (MRD), but still the risk of graft-versus-host disease (GvHD), and its high morbidity and mortality rate continue to impact the choice between HSCT versus immunosuppressive therapy (IST), as initial treatment in adults with SAA (Scheinberg *et al.,* 2012).

Post-transplant cyclophosphamide (PTCY) has become widely used as GvHD prophylaxis (Kanakry *et al.,* 2016), it has been used in MRD transplants and haploidentical transplants resulting in a lower incidence of acute and chronic GvHD (Luznik *et al.,* 2010; Kanakry *et al.,* 2014).

Since PTCY as anti- GvHD was studied in hematological malignancies, the studies performed on its role in adult SAA are few. So, in attempt to evaluate the effect of PTCY in adult Egyptian patients with SAA having HSCT from fully HLA-MRD and its effect on the outcome, regarding acute and chronic GvHD and OS, this work was designed.

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2. Patients and Methods:

Study design:

Sixty patients with SAA who were eligible for allogeneic HSCT, were recruited from Bone Marrow Transplantation Center of Nasser Institute, and Bone Marrow Transplantation Center and Hematology Unit of Internal Medicine Department, Tanta University Hospital (double center, prospective study). The duration of the study was from March 2020 till March 2022 (one year for patient's recruitment and the following year for follow up).
An informed consent was taken from all participants in the research, and the privacy of the data has been greatly considered.

These patients were divided into two groups: **Group I**: 30 patients received CY plus cyclosporine A (CsA) as an anti GvHD regimen. **Group II**: 30 patients (historical controls from our recorded data from 2010 till 2018) had received CsA and short-term methotrexate (MTX) as an anti GvHD regimen (the standard regimen) in this study.

Inclusion criteria: we included patients older than 18 years, who were diagnosed to have acquired SAA, and they had a fully HLA-MRD from whom peripheral stem cells were collected.

Exclusion criteria: we excluded those with pregnancy, HIV disease, active infections, refractory central nervous system disease and severe comorbidity.

After HLA typing for both the patient and potential donors were done, all patients had full history taking, and complete clinical examination, with grading of the patient performance status, according to Eastern cooperative oncology group (ECOG) scale. Complete blood count, bone marrow aspiration and biopsy, flow cytometry (CD55 and CD59), cytogenetic analysis, viral markers screening, and pregnancy test for females. Imaging: Echocardiography, abdominal ultrasound, computerized tomography of the chest and sinuses. Donors were subjected to pre-transplantation evaluation including the same hematological, biochemical, virology screen and echocardiography, in addition to bone marrow aspiration and cytogenetic study.

The transplant process:

All patients were isolated in single rooms, with laminar airflow, with strict disinfection measures, and prophylactic antimicrobials, with tendency to upgrade according to degree of infection, then received the conditioning regimen consisting of fludarabine-cyclophosphamide (FLU-CY); *Cyclophosphamide:* at a dose of 100 mg /Kg total dose, to be given as 25 mg/Kg /day, i.v. injection for four days (from day - 5 to day - 2 of stem cell infusion).*Fludarabine:* at a dose of 120 mg/m^2 total dose, to be given as 40 mg/m^2 /day, i.v. injection for three days (from day - 3 to day -1 of stem cell infusion).The cyclophosphamide was given concurrently with 2-Mercaptoethane sulfonate to protect against hemorrhagic cystitis. With GvHD prophylaxis either PTCY / CsA in group I; Cyclophosphamide: at a dose of 100 mg /Kg total dose to be given as 50 mg/Kg /day i.v. injection for two days (on day + 3 and day + 4 of stem cell infusion). Cyclosporine A administration: it was administered at a dose of 3 mg/kg/day i.v. in two divided doses from day + 5. Or MTX/ CsA in the historical control group: Methotrexate administration: bolus dose of 15 mg/m^2 on day + 1 then 10 mg/ m^2 on days +3, +6 and + 11, with folinic acid rescue; 15 mg/ m^2 intravenous infusion tds for just 24 hours the day after MTX injection. Cyclosporine A administration: a dose of 3 mg/kg/day i.v. in two divided doses from day - 1 and was changed to 12.5 mg/kg/day p.o. in two divided doses as soon as possible. Five days of subcutaneous G –CSF at a dose of 10 µg/kg were used for stem cells mobilization from the donors then peripheral blood stem cells were separated through leukapheresis. Typical target yield was collection of 2 to 5 × 10^6 / Kg CD34+ cells. Oral CsA for GvHD prophylaxis and prophylactic antimicrobial was continued for a minimum of 80 days post transplantation.

HSCT outcome was assessed according to the following parameters: Incidence and severity of infection, drugs' toxicities, acute & chronic GvHD, OS & event free survival (EFS).

Chimerism analysis:

Fluorescent in-situ hybridization was used to evaluate engraftment, degree of chimerism in patients at regular intervals by XY chromosome analysis in case of sex mismatch, and by PCR for variable number tandem repeats analysis in case of sex matching, at D+28 and D+56 post-transplant, using loci Apo B, YNZ, DIS 80, 33.1, 33.4, 33.6, and H Ras (Mahmoud *et al.,* 2015).

Graft failure:

Absence of neutrophil recovery, as well as only a transient engraftment of donor cells by day 28 after transplantation was considered as primary graft failure. while secondary graft failure was defined as ANC <0.5 x 10^9/L after initial neutrophil recovery (Runaas *et al.,* 2021).

GvHD assessment:

AGvHD was graded according to (the 1994 Consensus Conference on Acute GvHD Grading) (Przepiorka *et al.,* 1994). Classification of cGvHD was performed using the (National Institutes of Health Consensus Development Project Criteria) (Filipovich *et al.,* 2005) Corticosteroids were the first-line therapy for aGvHD (grade II–IV) and extensive cGvHD.

Outcome definitions:

OS was calculated from time of transplant till death from any cause. Event-free survival was defined as time to complete 1 year follow up in the absence of transplant related morbidity or death due to any cause.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** Significance of the obtained results was judged at the 5% level. Chi-square test to compare categorical variables between different groups. Fisher's Exact or Monte Carlo correction for chi-square when more than 20% of the cells have expected count less than 5. Kaplan-Meier Survival curve was used for the relation with overall survival and event free survival. Logistic Regression was used to detect the most affecting factor for acute and chronic GvHD.

3. Results

In our study we compared PTCY/CsA with CsA/MTX as GvHD prophylaxis regimens in adult Egyptian patients with SAA the performance status was significantly higher in group II in comparison to group I (P=0.002) as shown in table 1, this might be attributed to the earliest diagnosis and treatment now than before and the availability of more centers for transplant with shorter waiting lists. In comparison to stem cell dose, group II had a significant higher number of stem cells than group I (P=0.006).

Neutrophil engraftment also was significantly different between both groups, as neutrophil in group II engrafted faster in comparison to group I (p <0.001), while in group I primary graft failure occurred in one case and secondary graft failure occurred in one case and also poor graft function in one case, and none in group II, which might be attributed to the higher dose of stem cells in group II than group I and might indicate that PTCY has a role in delayed engraftment in group I.

Regarding Post-transplant complications: mucositis grade was significantly different in both groups, as group II patients had more severe mucositis than group I (^{MC}p= 0.045) as shown in table 2. This may be attributed to MTX use in group II and the lower performance status of group II than group I. Infection with klebsiella was found in blood cultures of 7 patients in group I, while it was absent in group II with significant difference between both groups, this might be attributed to the appearance of resistant strains.

As regard CMV reactivation cases number, titer, and day of PCR, it occurred only in two cases (6.7%) of group I, while in group II, it occurred in three cases (10%) with no statistically significant difference between both groups regarding number, titer of PCR and day of reactivation. (^{FE}*p=1) & (p value 0.603) & (p value 0.37)* respectively

Table 1: Patients' and donors' characteristics in both groups.

	PTCY/CsA N (%)	**CsA/MTX N (%)**	**P-value**
Sex			0.152
Male	19 (63)	24 (80)	
Female	11 (37)	6 (20)	
Age mean ± SD.	29.73 ± 10.04	29.87 ± 9.17	0.957
BMI (kg/m^2) mean ± SD.	29.10 ± 5.70	27.0 ± 6.85	0.201
Comorbidity	15 (50)	18 (60)	0.436
CLD	1 (3.3)	4 (13.3)	
Obesity	13 (43.3)	10 (33.3)	
DM	1 (3.3)	0 (0)	
HCV	2 (6.7)	2 (6.7)	
Congenital single kidney	0 (0)	1 (3.3)	
Valve disease	0 (0)	3 (10)	
Nephrectomy	1 (3.3)	0 (0)	
PS (ECOG)			0.002*
0	12 (40)	2 (7)	
1	13 (43)	12 (40)	
2	5 (17)	16 (53)	
Blood group matching			0.438
Matching	13 (43.3)	16 (53.3)	
Non matching	17 (56.7)	14 (46.7)	
Sex matching			0.039*
Matching	11 (36.7)	19 (63.3)	
Non matching	19 (63.3)	11 (36.7)	
Female to Male	12 (40)	8 (26.7)	0.104
Male to Female	7 (23.3)	3 (10)	
Degree of ABO mismatch			0.340
Minor	8 (26.7)	5 (16.7)	
Major	5 (16.7)	3 (10)	
Matched	14 (46.7)	21 (70)	
Bidirectional	3 (10)	1 (3.3)	
D-R pair CMV status			0.168
Neg. Neg.	3 (10)	0 (0)	
Pos. Pos.	22 (73.3)	26 (86.7)	
Neg. Pos.	3 (10)	4 (13.3)	
Pos. Neg.	2 (6.7)	0 (0)	
Hemoglobin (gm/dl) mean ± SD.	7.39 ± 1.59	7.79 ± 2.43	0.454
Platelets. x10^3/mm^3 median (range)	18 (2– 164)	15 (4 – 195)	0.437
WBCs x10^3 /mm^3 median (range)	2.45 (0.7 – 8.4)	2.85 (0.7 – 7.3)	0.437
BM Cellularity median (range)	10 (5 – 25)	20 (0 – 25)	0.087
Serum Ferritin (ng/ml) median (range)	1500 (153 – 3000)	1293 (23 –7726)	0.469
Diagnosis-Transplantation Lag (month) median (range)	6.5 (3 – 24)	6 (3 – 15)	0.250
Dose of stem cells CD^{34} x 10^6/Kg mean ± SD.	5.48 ± 1.51	8.02 ± 4.56	0.006*
Antimicrobial duration(days) median (range)	14 (7 – 45)	12 (4 – 24)	0.497
Transfusion RBCs (unit) median (range)	5 (1 – 14)	6 (0 – 21)	0.187
Transfusion Plat. (unit) median (range)	30 (6 – 144)	30 (0 – 102)	0.743
Hospital stays (days) median (range)	29 (12 – 55)	31 (14 – 80)	0.097
Sex (donor)			0.194
Male	14 (47)	19 (63)	
Female	16 (53)	11 (37)	
Age (donor) Mean ± SD.	30.20 ± 11.19	29.53 ± 12.16	0.826

P p value for comparing between the two studied groups, Statistically significant at $p \leq 0.05$, BMI Body mass index, PS Performance status, ECOG Eastern Cooperative Oncology Group, D-R donor- recipient.

Table 2: Post transplantation complications in both groups.

GvHD prophylaxis	PTCY/CsA N (%)	CsA/MTX N (%)	P-value
Death before engraftment	8 (27)	5 (17)	0.347
Day of ANC >500 (cell/ mm^3) mean ± SD.	(n = 22) 16.0 ±2.89	(n = 25) 12.08 ±2.60	<0.001
Day of Platelets >20,000 (cell/ mm^3) mean ± SD.	(n = 20) 16.25 ±5.19	(n = 23) 15.0 ±3.21	0.341
Graft Failure no.	3(10)	0(0)	0.0237
Primary graft failure	1(3.3)	0(0)	1.000
Secondary graft failure	1(3.3)	0(0)	1.000
Poor graft function	1(3.3)	0(0)	1.000
Mucositis Grade			0.045
0	3 (10)	0 (0)	
1	5 (16.7)	2 (6.7)	
2	14 (46.7)	11 (36.7)	
3	8 (26.7)	17 (56.7)	
Infection			0.111
No	15 (50)	22 (73.3)	
Bacterial	14 (46.7)	8 (26.7)	
Fungal	1 (3.3)	0 (0)	
Blood Culture Type			0.011
Klebsiella pneumoniae	7 (23.3)	0 (0)	
VOD	0 (0)	0 (0)	–
Hemorrhagic cystitis	4 (13.3)	0 (0)	0.112
Hemorrhage	2 (6.7)	1 (3.3)	1.000
aGvHD			
Number	2 (6.7)	11 (36.7)	0.005
Grade			0.002
I	1 (3.3)	0 (0)	
II	1 (3.3)	3 (10)	
III	0 (0)	8 (26.7)	
VI	0 (0)	0 (0)	
Site			
Skin	0 (0)	8 (26.7)	0.005
Liver	0 (0)	1 (3.3)	1.000
GIT	2 (6.7)	8 (26.7)	0.038
cGvHD			
Number	3 (10)	17 (56.7)	0.001
Extension			<0.001
Limited	1 (3.3)	7 (23.3)	
Extensive	2 (6.7)	10 (33.3)	
Onset			<0.001
Denovo	3 (10)	7 (23.3)	
Quiescent/ Progressive	0 (0)	10 (33.3)	
Site			
Skin	1 (3.3)	12 (40)	0.001
Liver	0 (0)	6 (20)	0.024
Git	0 (0)	0 (0)	–
Eye	1 (3.3)	7 (23.3)	0.052
Oral	0 (0)	7 (23.3)	0.011
Lung	2 (6.7)	3 (10)	1.000
Day of cGvHD mean ± SD.	(n = 3) 197.0 ± 53.56	(n = 13) 298.2 ± 31.8	0.594
CMV reactivation	2 (6.7)	3 (10)	1.000
Day (mean ± SD.)	48.50 ± 0.71	56.67 ± 10.41	0.370
Titre of PCR (mean ± SD.)	16950 ± 16192.8	10600 ± 9245.5	0.603

P p value for comparing between the two studied groups, Statistically significant at $p \leq 0.05$, *aGvHD* Acute graft versus host disease, *cGvHD* Chronic graft versus host disease, *ANC* Absolute neutrophilic count, *CMV* Cytomegalovirus

As regard acute GvHD, group I was significantly lower than group II regarding number of cases, grade of GvHD and site of involvement, having only two patients (6.7%), one patient had grade I, and the other one had grade II, only involving GIT, and both patients responded well to steroids. While group II had eleven patients (36.7%) had aGvHD, three of grade II, and eight of grade III, involving skin, liver and GIT, ten patients showed partial response to steroid, however they progressed to chronic GvHD and one patient died of acute complications, as shown in table 3. This lower incidence of aGvHD in group I, might be attributed to the use PTCY in this group.

Table 3: CsA complications & IST therapy in both groups

CsA complications & IST therapy	PTCY/CsA N (%)	CsA/MTX N (%)	P-value
Neurologic toxicity	1 (3.3)	4 (13.3)	0.353
Hemolysis	2 (6.7)	3 (10)	1.000
Renal toxicity with sr.cr < 2 (mg/dl)	5 (16.7)	2 (6.7)	0.424
Renal toxicity with sr.cr >2 (mg/dl)	3 (10)	14 (46.7)	0.002*
IST therapy			0.010*
Still >1 year	4 (13.3)	11 (36.7)	
Stopped CsA	17 (56.7)	6 (20)	
Up to death	9 (30)	13 (43.3)	

P p value for comparing between the two studied groups, * Statistically significant at p ≤ 0.05, *CSA* Cyclosporine A.

Table 4: Follow up, Death rate, time and cause in both groups

Follow up, Death rate, time and cause	PTCY/CsA N (%)	CsA/MTX N (%)	P-value
Follow Up	(n = 21)	(n = 17)	0.492
Regular	19 (63.3)	17 (56.7)	
Lost follow up	2 (6.7)	0 (0)	
Death			
Number	9 (30)	13 (43.3)	0.284
Day of death median (range)	11 (1 – 50)	61 (5 – 263)	0.186
Cause of death			
Septic shock	8 (88.9)	8 (61.5)	0.333
aGvHD	0 (0)	2 (15.4)	0.494
CMV infection	0 (0)	2 (15.4)	0.494
Diffuse alveolar hemorrhage	0 (0)	1 (7.7)	1.000
Sudden arrest	1 (11.1)	0 (0)	0.409

Unfortunately, nine patients died in group I, eight of them with septic shock, which may be due to the emergence of resistant strains of bacteria as mentioned above. and one suddenly arrested. While in group II, thirteen patients died, eight of them with septic shock, two with aGvHD, two with CMV infection, and one patient with diffuse alveolar hemorrhage, with no statistically significant difference between the two groups.

In table 5, univariate logistic regression analysis showed that only type of GvHD prophylaxis regimen (CsA/MTX) affected the rate of occurrence of aGvHD (p =0.011). While the type of GvHD prophylaxis regimen (CsA/MTX), PS score by ECOG scale and the previous occurrence of aGvHD affected the rate of occurrence of cGvHD (P=0.001), (P=0.008) and (P <0.001), respectively. But with multivariate logistic regression, only the previous occurrence of aGvHD affected the occurrence of cGvHD (P=0.002) with HR (95%C.I) of 20.462.

In our study, event free survival curve showed similar rates in both groups, while OS was superior in group I (70%) than group II (56.7%) but the difference was not statistically significant between both groups as shown in figure 1.

Table 5: Univariate and multivariate Logistic regression analysis for the parameters affecting acute and chronic GvHD

GvHD Risk factors	Univariate for aGvHD (n = 13 vs. 47) HR [95%] CI *P*-value	Univariate for cGvHD (n = 20 vs. 40) HR [95%] CI *P*-value	Multivariate for cGvHD HR [95%] CI *P*-value
GvHD Prophylaxis			
PTCY/CsA	1.000	1.000	
CsA/MTX	8.105 (1.612 –40.766) 0.011*	11.769 (2.919 –47.458) 0.001*	4.642 (0.854 –25.228) 0.076
Recipient Age (years)	0.987 (0.924 –1.055) 0.706	0.982 (0.927 –1.041) 0.544	
Male recipient	6.194 (0.738 –51.980) 0.093	1.926 (0.536 –6.926) 0.316	
Blood group (O+ve)	1.473 (0.409 –5.300) 0.553	1.758 (0.566 –5.453) 0.329	
PS(ECOG)	1.559 (0.665 –3.654) 0.307	3.156 (1.345 –7.404) 0.008*	2.701 (0.835 –8.737) 0.097
CMV Ig G of the recipient	1.116 (0.114 –10.944) 0.925	2.111 (0.220 –20.245) 0.517	
Female donor	1.575 (0.458 –5.411) 0.471	1.353 (0.461 –3.975) 0.582	
Age of donor (years)	1.008 (0.956 –1.063) 0.770	0.975 (0.928 –1.024) 0.307	
DR sex mismatched	1.818 (0.518 –6.382) 0.351	1.0 (0.342 –2.926) 1.0	
MTF	0.352 (0.040 –3.068) 0.344	0.444 (0.085 –2.323) 0.336	
FTM	3.051 (0.862 –10.799) 0.084	1.556 (0.507 –4.774) 0.440	
Degree of ABO			
Minor®	1.000	1.00	
Major	1.833 (0.204 –16.512) 0.589	1.600 (0.270 –9.490) 0.605	
Matched	1.630 (0.297 –8.927) 0.574	0.640 (0.168 –2.436) 0.513	
Bidirectional	1.833 (0.121 –27.797) 0.662	0.533 (0.043 –6.655) 0.625	
CMV Ig G of the donor	1.128 (0.209 –6.101) 0.889	1.202 (0.275 –5.247) 0.807	
D-R pair CMV status			
-ve-ve	0.0 (0.0 –.) 0.999	0.0 (0.0 –.) 0.999	
+ve+ve	0.789 (0.180 –3.471) 0.754	1.000 (0.261 –3.826) 1.000	
-ve+ve	1.527 (0.260 –8.958) 0.639	1.588 (0.319 –7.900) 0.572	
+ve-ve	3.833 (0.223 –65.853) 0.354	2.053 (0.122 –34.628) 0.618	
Dose of stem cell CD34 x 106/Kg Mean ± SD.	1.014 (0.857 –1.200) 0.868	0.974 (0.834 –1.138) 0.740	
Pervious aGvHD		23.222 (4.360 –123.692) <0.001	20.462 (2.894 –144.664) 0.002*

HR Hazard ratio, *C.I* Confidence interval,* Statistically significant at p ≤ 0.05, *PS* performance status, *CMV IgG* Cytomegalovirus immunoglobulin G, *aGvHD* Acute graft versus host disease, *cGvHD* Chronic graft versus host disease, *D-R* donor- recipient, *MTF* Male to female, *FTM* Female to male.

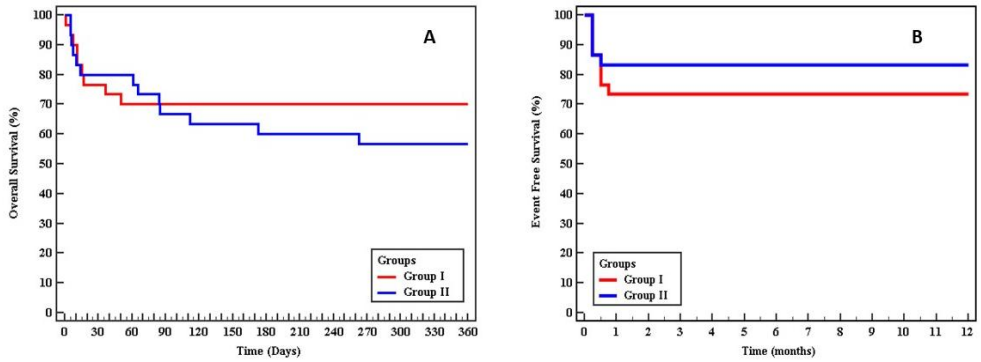

Fig. 1: Kaplan Meier analysis for estimation of the OS and EFS of both groups.

A: Group I had slight superiority in OS than group II with no statically significant difference between both groups (*p= 0.385*). **B:** No statistically significant difference between both groups regarding EFS(*P=0.379*).

4. Discussion

Aplastic anemia is still considered a huge burden and a fatal condition not only for its complications due to severe pancytopenia but also for its possible transformation into leukemia (Mielcare *et al.,* 2016). HSCT using an MSD is the first line in a young patient with SAA (Scheinberg *et al.,* 2012) but still GvHD is considered the biggest rock in the way of its full success (Mielcare *et al.,* 2016). Antithymocyte globulin is used to reduce GvHD in patients receiving a PBSC transplant, but its high cost is limiting its use in the developing countries (George *et al.,* 2018).

PTCY was first used to lower GvHD rate and to have a better engraftment after HLA-haploidentical BMT (Brunstein *et al.,* 2011; McCurdy *et al.,* 2017; Bashey et al., 2017). Its success in lowering severe aGvHD and cGvHD in haploBMT made it a possible player in HLA matched BMT (Luznik *et al.,* 2010). However, few data are present regarding its use in MRD and MUD transplants (El Fakih *et al.,* 2020).

PTCY was evaluated first in hematological malignancies in MRD and MUD, multiple studies using multiple conditioning regimens and using PTCY alone or in addition to other immunosuppressive drugs were done (Luznik *et al.,* 2010; Solomon *et al.,* 2014; Kanakry *et al.,* 2014; Alousi *et al.,* 2015; O'Donnell *et al*., 2016; Carnevale-Schianca *et al.,* 2017, 2021; Bradstock *et al.,* 2015).

Most of them concluded that PTCY lower acute and chronic GvHD significantly (Luznik *et al.,* 2010; Solomon *et al.,* 2014; O'Donnell et al., 2016; Carnevale-Schianca *et al.,* 2021), while other studies showed no difference (Kanakry *et al.,* 2014) or even higher rate of GvHD (Alousi *et al.,* 2015; Bradstock *et al.,* 2015).

All the previous studies were performed on patients with hematological malignancies. On the other hand, George *et al.* (2018) used PTCY as a single agent in GvHD prophylaxis in matched donors of SAA patients.

They conducted their study on 30 patients with fully identical HLA -MRD using peripheral blood stem cells as a graft source. Patients received only PTCY at day +3 and + 4 post-transplants after conditioning regimen, using FLU, CY, and total body irradiation.

They reported higher percentage of both aGvHD and cGvHD of (22%) for both, while we reported only (6.7%) and (10%), respectively. This may be attributed to the fact that we used CsA in addition to PTCY starting at day +5 forward, as multiple studies concluded that adding calcineurin inhibitors may further reduce GvHD incidences after blood cell grafts ((Mielcare *et al.,* 2016; Carnevale-Schianca *et al.,* 2017, 2021; Robinson *et al.,* 2014).

They compared their results with two other studies used CsA/MTX as GvHD prophylaxis as control, which also showed comparable results to our historical control group as acute and chronic GvHD occurred in (53.8%) and (63.6%) in their control and (36.7%) and (56.7%) in our historical control, respectively.

OS was also comparable as it was 65.9 % for their study and 70% in ours. Finally, they concluded that PTCY was associated with a significantly lower incidence of acute and chronic GvHD compared with CsA/MTX.

Another study had investigated PTCY in hematological malignancies and SAA (Mahmoud *et al.,* 2020)], where there were only 13 patients with SAA, both young and adults, with age ranging from 4 to 47 years old, and received PTCY and CsA as GvHD prophylaxis with follow up for 1 year, which resulted in lower incidence of acute and chronic GvHD, with OS of (78.6 %), which is comparable to this study.

There were some limitations in the present study that must be addressed. First, the small number of patients enrolled was inadequate to obtain conclusive data. Second, short follow-up period. But despite these limitations, our data may help optimizing allogeneic HSCT GvHD prophylaxis regimens for SAA specially in low-income countries.

Conclusions

The addition of CY to CsA as an anti-GvHD regimen in patients having SAA undergoing HSCT is associated with good engraftment rate, positive disease outcome, tolerable toxicity and showed a significant lower rate of both acute and chronic GvHD with superior OS than old regimen and its use may help lowering transplant related economic and social burden and the use of other expensive immunosuppressive drugs.

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All authors reviewed and finally approved the manuscript. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Compliance with Ethical Standards:

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards**.**

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The authors declare that they have none to report.

References

Alousi, A.M., J.E. Brammer, R.M. Saliba, B. Andersson, U. Popat, 2015. Hosing C, et al. Phase II trial of graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide after reduced-intensity busulfan/fludarabine conditioning for hematological malignancies. 21(5):906-12. https://doi.org/10.1016/j.bbmt.2015.01.026

Bashey, A., M.J. Zhang, S.R. McCurdy, A.S. Martin, T. Argall, C. Anasetti, et al., 2017. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell–replete haploidentical donor transplantation using post-transplant cyclophosphamide. 35(26):3002. 10.1200/JCO.2017.72.8428

Bradstock, K.F., I. Bilmon, J. Kwan, K. Micklethwaite, E. Blyth, S. Deren, et al., 2015. Single-agent high-dose cyclophosphamide for graft-versus-host disease prophylaxis in human leukocyte antigen–matched reduced-intensity peripheral blood stem cell transplantation results in an

unacceptably high rate of severe acute graft-versus-host disease. 21(5):941-4. https://doi.org/10.1016/j.bbmt.2015.01.020

Brodsky, R.A. and R.J. Jones, 2005. Aplastic anaemia. The Lancet, 365(9471): 1647-1656. https://doi.org/10.1016/S0140-6736(05)66515-4

Brunstein, C.G., E.J. Fuchs, S.L. Carter, C. Karanes, L.J. Costa, J. Wu, et al., 2011. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. 2011;118(2):282-8. https://doi.org/10.1182/blood-2011-03-344853

Carnevale-Schianca, F., D. Caravelli, S. Gallo, V. Coha, L. D'Ambrosio, E. Vassallo, et al., 2017. Post-transplant cyclophosphamide and tacrolimus–mycophenolate mofetil combination prevents graft-versus-host disease in allogeneic peripheral blood hematopoietic cell transplantation from HLA-matched donors. 23(3):459-66. https://doi.org/10.1016/j.bbmt.2016.12.636

Carnevale-Schianca, F., D. Caravelli, S. Gallo, P. Becco, L. Paruzzo, S. Poletto, et al., 2021. Post-Transplant Cyclophosphamide and Tacrolimus—Mycophenolate Mofetil Combination Governs GVHD and Immunosuppression Need, Reducing Late Toxicities in Allogeneic Peripheral Blood Hematopoietic Cell Transplantation from HLA-Matched Donors. 10(6):1173. https://doi.org/10.3390/jcm10061173

El Fakih, R., S.K. Hashmi, S.O. Ciurea, L. Luznik, R.P. Gale, M. Aljurf, 2020. Post-transplant cyclophosphamide use in matched HLA donors: a review of literature and future application. Bone Marrow Transplantation volume 55, pages40–47. https://doi.org/10.1038/s41409-019-0547-8

Filipovich, A.H., D. Weisdorf, S. Pavletic, G. Socie, J.R. Wingard, S.J. Lee, et al., 2005. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. 11(12):945-56. https://doi.org/10.1016/j.bbmt.2005.09.004

George B, P. Nisham, A.J. Devasia, U. Kulkarni, A. Korula, K.M. Lakshmi, et al. Post-transplant cyclophosphamide as sole graft-versus-host disease prophylaxis is feasible in patients undergoing peripheral blood stem cell transplantation for severe aplastic anemia using matched sibling donors. 2018;24(3):494-500. https://doi.org/10.1016/j.bbmt.2017.10.034

Kanakry, C.G., E.J. Fuchs and L. Luznik, 2016. Modern approaches to HLA-haploidentical blood or marrow transplantation, Nature Reviews Clinical Oncology volume 13, pages10–24. https://doi.org/10.1038/nrclinonc.2015.128

Kanakry, C.G., H.L. Tsai, J. Bolaños-Meade, B.D. Smith, I. Gojo, J.A. Kanakry, et al., 2014. Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. 124(25):3817-27. https://doi.org/10.1182/blood-2014-07-587477

Kanakry, C.G., P.V. O'Donnell, T. Furlong, M.J. De Lima, W. Wei, M. Medeot, et al., 2014. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. 2014;32(31):3497. 10.1200/JCO.2013.54.0625

Luznik, L., J. Bolaños-Meade, M. Zahurak, A.R. Chen, B.D. Smith, R. Brodsky, et al., 2010. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. 2010;115(16):3224-30. https://doi.org/10.1182/blood-2009-11-251595

Mahmoud, H.K., A.M. Elhaddad, O.A. Fahmy, M.A. Samra, R.M. Abdelfattah, Y.H. El-Nahass, Fathy GM, Abdelhady MS. Allogeneic hematopoietic stem cell transplantation for non-malignant hematological disorders. Journal of Advanced Research. 2015 May 1;6(3):449-58. https://doi.org/10.1016/j.jare.2014.11.001

Mahmoud, H.K., G.M. Fathy, A. Elhaddad, O.A. Fahmy, M. Abdel-Mooti, R. Abdelfattah, et al. 2020. The Use of Post-transplantation Cyclophosphamide in Peripheral Blood HLA-matched Stem Cell Transplantation as Graft-versus-host Disease Prophylaxis in Patients With Malignant or Non-malignant Hematologic Disorders: A Single-center Experience of 52 Patients. 2020;20(10):677-84. https://doi.org/10.1016/j.clml.2020.04.021

McCurdy, S.R., Y.L. Kasamon, C.G. Kanakry, J. Bolaños-Meade, H.L.Tsai, M.M. Showel et al., 2017. Comparable composite endpoints after HLA-matched and HLA-haploidentical transplantation with post-transplantation cyclophosphamide. 102(2):391.

10.3324/haematol.2016.144139

Mielcarek, M., T. Furlong, P.V. O'Donnell, B.E. Storer, J.S. McCune, R. Storb, et al., 2016. Posttransplantation cyclophosphamide for prevention of graft-versus-host disease after HLA-matched mobilized blood cell transplantation. 127(11):1502-8. https://doi.org/10.1182/blood-2015-10-672071

O'Donnell, P.V., M. Eapen, M.M. Horowitz, B.R. Logan, A. DiGilio, C. Brunstein, et al., 2016. Comparable outcomes with marrow or peripheral blood as stem cell sources for hematopoietic cell transplantation from haploidentical donors after non-ablative conditioning: a matched-pair analysis. 51(12):1599-601. https://doi.org/10.1038/bmt.2016.215

Przepiorka, D., D. Weisdorf, P. Martin, H. Klingemann, P. Beatty, J. Hows, et al. 1994. Consensus conference on acute GVHD grading. 15(6):825-8. - Abstract - Europe PMC

Scheinberg, P., O. Nunez, B. Weinstein, P. Scheinberg, C.O. Wu and N.S.J.B. Young, 2012. The Journal of the American Society of Hematology. Activity of alemtuzumab monotherapy in treatment-naive, relapsed, and refractory severe acquired aplastic anemia. 2012;119(2):345-54. https://doi.org/10.1182/blood-2011-05-352328

Solomon, S.R., M. Sanacore, X. Zhang, S. Brown, K. Holland, L.E. Morris, et al., 2014. Calcineurin inhibitor–free graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide and brief-course sirolimus following reduced-intensity peripheral blood stem cell transplantation. 20(11):1828-34. https://doi.org/10.1016/j.bbmt.2014.07.020

Robinson, D., G. Schulz, R. Langley, K. Donze, K. Winchester, C.Rodgers, 2014.. Evidence-based practice recommendations for hydration in children and adolescents with cancer receiving intravenous cyclophosphamide. 31(4):191-9. https://doi.org/10.1177/1043454214532024

Runaas L, P. Hari, S. Chhabra, 2021. Graft Failure. Blood and Marrow Transplant Handbook: Springer; 2021. p. 691-700. https://doi.org/10.1007/978-3-030-53626-8_42