Original Research Article

Post-transplant Cyclophosphamide is a valid option for GVHD Prophylaxis in both HLA-matched and mismatched Allogeneic transplants

Abstract

Despite improvement in transplantation techniques and supportive care, graft versus host disease (GVHD) remains a major cause of post-transplant morbidity and mortality. Advances in immunosuppressive regimens have reduced the incidence and severity of acute GVHD; with no such impact on chronic GVHD. In addition to the considerable toxicity of calcineurin inhibitors (CNI)-based regimens[1]. We compared the safety and efficacy of post allogeneic transplant cyclophosphamide as GVHD prophylaxis versus CNI prophylaxis. This study included 63 patients (2 groups) admitted to Hematology unit, Maadi Armed Forces Medical Compound, Cairo for allogeneic stem cell transplantation for hematological malignancies. Group I received post-transplant cyclophosphamide prophylaxis (50 mg /kg/day) , while group II received CNI based prophylaxis. Patients were followed for 6 months for acute and chronic GVHD, infections, and drug toxicities. Most patients successfully underwent hematopoietic reconstitution with no significant difference between both groups in time to hematological recovery. Hepatic and renal toxicities were more common in group II than group I (78.6% vs. 30.8%, ***P***=0.002) and (60% vs. 20.5%, ***P***=0.022) respectively. No significant difference between both groups in the incidence of CMV reactivation or acute and chronic GVHD. Disease free survival and overall survival were not significantly different between both groups either in matched or mismatched transplants, with a trend for better survival in group I than group II (4 vs. 1.4 months, ***P***=0.087). We concluded that post-transplant cyclophosphamide for GVHD prophylaxis is safe , effective and valid option in allogeneic stem cell transplantation, with a different toxicity profile compared with CNI regimens.[2]

Keywords: cyclophosphamide; allogeneic stem cell transplantation; GVHD

Introduction

An optimal graft versus host disease (GVHD) prevention regimen must be effective, well tolerated, permit rapid immune reconstitution, and preserve the GVL effect.[3]Despite improvements in transplantation techniques and supportive care, GVHD remains a major source of post-transplantation morbidity and mortality. Although advances in immunosuppressive regimens have had some impact on the incidence and severity of acute GVHD, they have had little impact on the incidence and severity of chronic GVHD. [4]

Improvement in GVHD prophylaxis can be achieved by either a reduction in the incidence and severity of acute GVHD or a reduction in complications associated with immunosuppressive therapy. Ultimately, the goal is to achieve long-term disease-free survival with full immune reconstitution without acute or chronic GVHD.

The current pharmacologic prophylaxis, employing different combinations of methotrexate, mycophenolic acid, calcineurin inhibitors, mammalian target of rapamycin (mTor) inhibitors, and antithymocyte globulin all aim to indiscriminately inhibit or eliminate T cells and, therefore, fall short of meeting the above requisites.[1]

In this study, we assessed the safety and efficacy of cyclophosphamide as GVHD prophylaxis in allogeneic stem cell transplant and its effect on acute GVHD, chronic GVHD, infections and drug toxicities.

Patients and Methods

The work was conducted on 63 Egyptian patients with malignant hematological diseases (including leukemias, lymphomas, and Multiple Myeloma) who attended Hematology unit in Maadi Armed Forces Medical Compound in Cairo for allogeneic stem cell transplantation. The study population comprised of 46 male (73%) and 17 female (27%) patients, with a male to female ratio of 2.7. After obtaining a written informed consent from all patients, they were divided into two groups; Group I comprised of 39 patients who received reduced intensity conditioning followed by allogeneic stem cell transplant and post-transplant high dose cyclophosphamide (50 mg /kg/day) at day +3 and day +4 as GVHD prophylaxis. Group II consisted of 24 patients who received the same conditioning as group I but with post allogeneic stem cell CNI based GVHD prophylaxis. Patients were followed for 6 months and assessed for acute GVHD, infections, drug toxicities and chronic GVHD.

Statistical Analysis

Data were analysed using ***SPSS*** program (SPSS, Inc, Chicago, IL) version 20. Qualitative data were presented as frequency and percentage. Chi square or Fisher's exact tests were used to compare groups. Quantitative data were presented as mean, standard deviation, median and range. The quantitative data were examined by Kolmogorov Smirnov test for normality. For comparison between two groups; student t-test, and Mann-whitney test (for non-parametric data) were used. For comparison between more than two groups; one way ANOVA or Krusskal Wallis test (for non-parametric data) were used. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. Cox regression analysis was used to assess prediction of overall survival (OS) and disease-free survival (DFS) in all studied patients. P-values of <0.05 were considered significant.

Results

Patient characteristics are summarized in Table 1.

Table 1. Patient's demographics and diagnosis

	All patients (*n*=63)	**Group 1 (Post-transplant cyclo) (*n*=39)**	**Group 2 (Conventional GVHD prophylaxis) (*n*=24)**	***P* value**
Age; Mean (± SD)		35.3 (± 11.3)	38.0 (±11.1)	0.508
Sex; Male (n %): Female (n %):	46 (73.0 %) 17 (27.0 %)	29 (74.4 %) 10 (25.6 %)	17 (70.8 %) 7 (29.2 %)	0.759
Diagnosis: Acute leukemia: Other than acute leukemia:	51 (81.0 % 12 (19.0 %)	34 (87.2 %) 5 (12.8 %)	17 (70.8 %) 7 (29.2 %)	0.102
HLA Groups: Matched siblings: Haploidentical: Not matched:	44 (69.8 %) 13 (20.6 %) 6 (9.5 %)	25 (64.1 %) 13 (33.3 %) 1 (2.6 %)	19 (79.2 %) 0 (0.0 %) 5 (20.8 %)	0.001

Engraftment Characteristics

The majority of patients successfully underwent hematopoietic reconstitution. The median time to recovery of total leucocytic count (TLC) was 14 days and median time to platelets recovery was 12 days. No significant difference was found between both groups in time to hematological recovery (Table 2).

Table 2. TLC and PLT recovery

	All patients (*n*=63)	**Group 1 (Post-transplant cyclo) (*n*=39)**	**Group 2 (Conventional GVHD prophylaxis) (*n*=24)**	***P* value**
TLC Recovery (Days): Median (IQR)	14 (5)	14 (5)	13 (30)	0.812 [2]
PLT Recovery (Days): Median (IQR)	12 (166)	13 (37)	12 (164)	0.526 [2]

Toxicity

The most common post-transplant toxicity was hepatic toxicity which was reported in 43.4% of patients. Hepatic toxicity was more common in group 2 (conventional GVHD prophylaxis) 78.6% vs. 30.8% in group 1 which was statistically significant (P=0.002). Other common toxicities were renal toxicity which was also higher in group 2 (60% vs. 20.5% in group 1, P=0.022), and hemorrhagic cystitis (16.2% in group 1 vs. no patient in group 2, P=1.0). The incidence of CMV reactivation didn't differ between both groups (35.1% in group I vs. 28.6% in group II, P=1.0). Regarding GVHD, the overall incidence of acute GVHD was 18.8%, and for chronic GVHD was 22.9%. There was no statistically significant difference in incidence of either acute or chronic GVHD between both groups (Table 3).

Table 3. Acute and chronic GVHD.

	All patients (*n*=63)	**Group 1 (Post-transplant cyclo) (*n*=39)**	**Group 2 (Conventional GVHD prophylaxis) (*n*=24)**	***P* value**
aGVHD Grade: N (%) - **No:** - **Mild:** - **Moderate:** - **Severe:**	 39 (81.2 %) 3 (6.2 %) 1 (2.1 %) 5 (10.4 %)	 31 (79.5 %) 3 (7.7 %) 1 (2.6 %) 4 (10.3 %)	 8 (88.9 %) 0 (0.0 %) 0 (0.0 %) 1 (11.1 %)	0.799

cGVHD Grade: N (%) - **No:** - **Mild:** - **Moderate:** - **Severe:**	 37 (77.1 %) 4 (8.3 %) 2 (4.2 %) 5 (10.4 %)	 29 (74.4 %) 3 (7.7 %) 2 (5.1 %) 5 (12.8 %)	 8 (88.9 %) 1 (11.1 %) 0 (0.0 %) 0 (0.0 %)	0.597

Survival Results

Regarding survival outcomes, DFS was not significantly different between both groups (median not reached in group I vs. 35.4 months in group II, (P=0.298). The same result was noticed when comparing DFS in matched and mismatched transplants (figures 1-3).

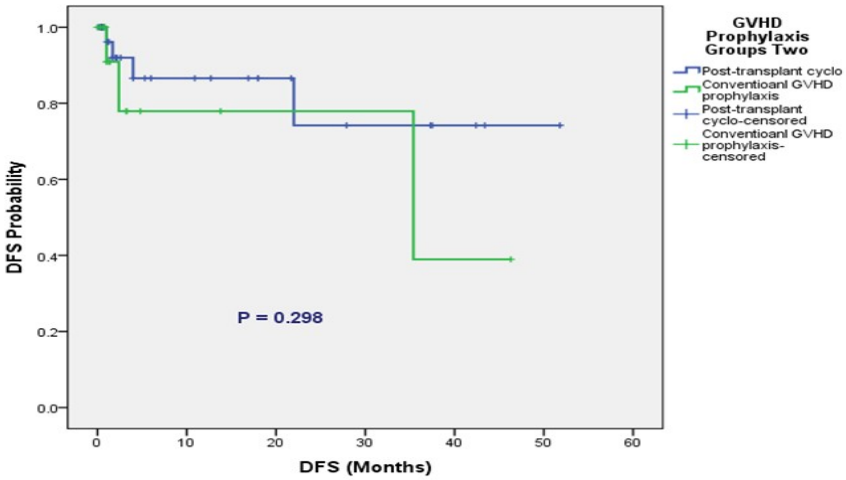

Figure 1. Disease free survival between both groups

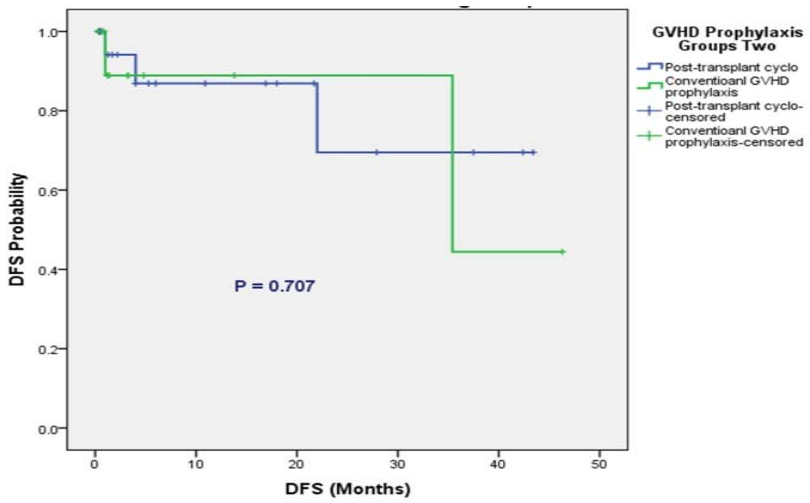

Figure 2. Disease free survival in matched sibling transplant

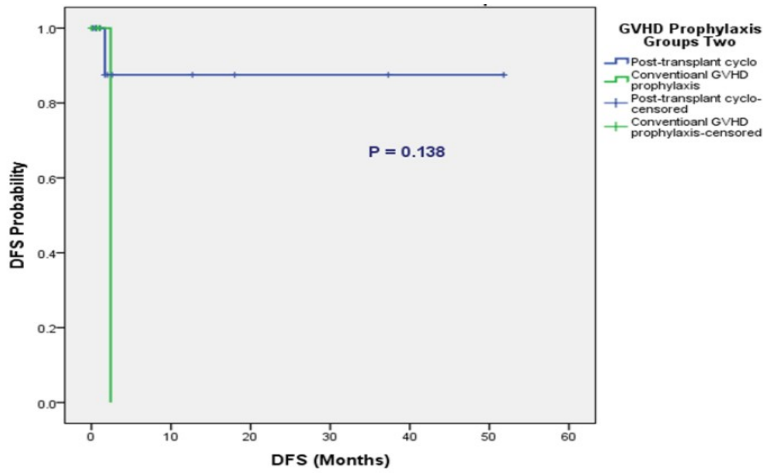

Figure 3. Disease free survival in mismatched transplant

Similarly there was no statistically significant difference regarding OS, although there was a trend toward better survival in group I compared to group II (4 vs. 1.4 months, P=0.087). This observation is applied again in both matched and mismatched transplant comparisons (figures 4-6).

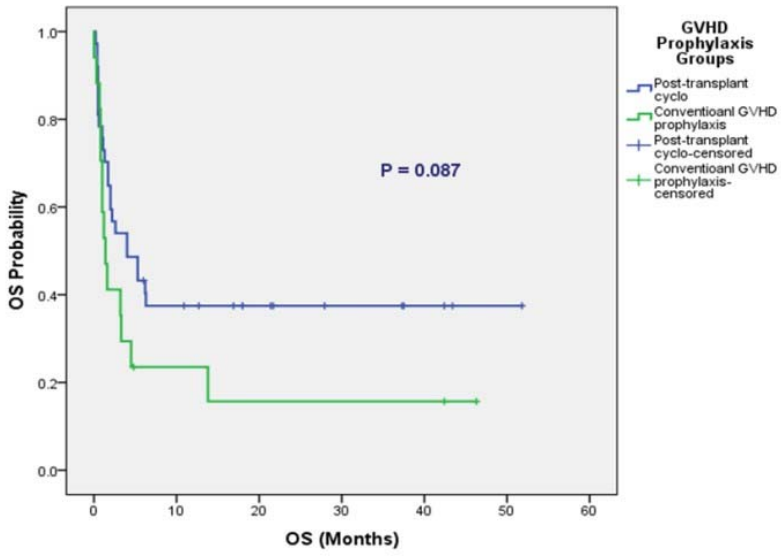

Figure 4. Overall survival between both groups

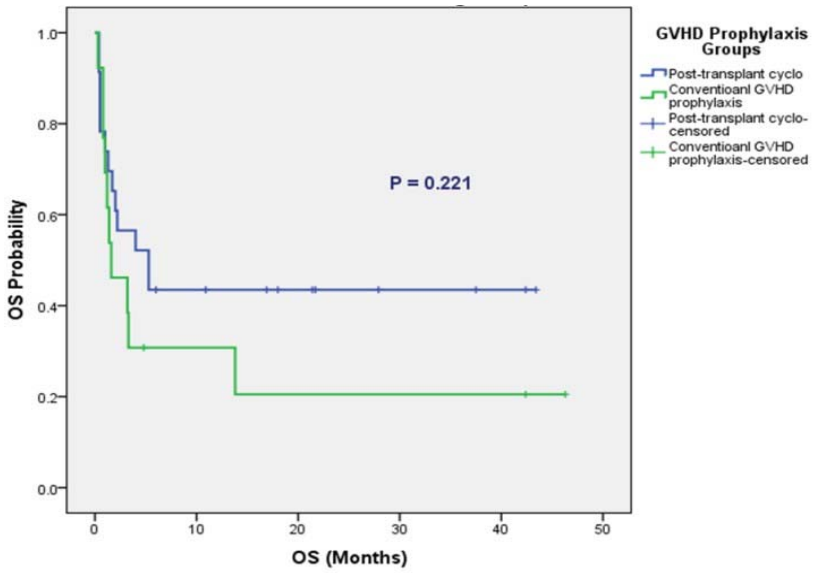

Figure 5. Overall survival in matched sibling transplant

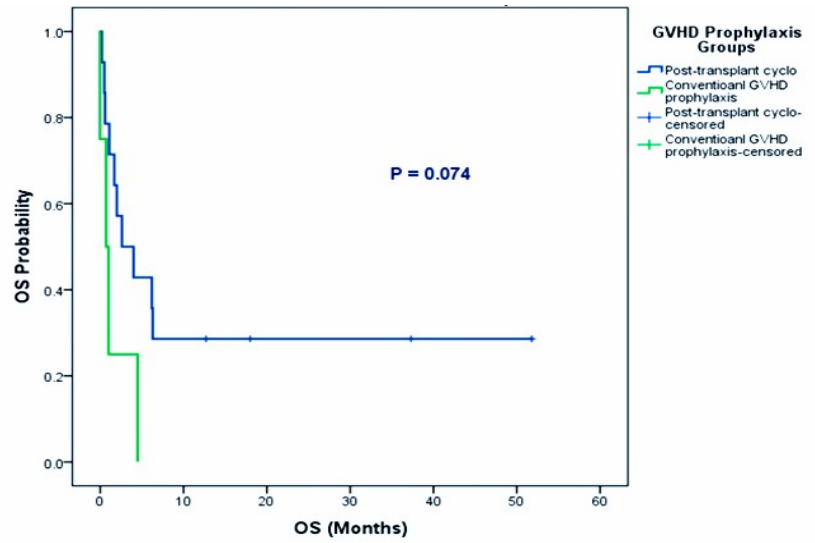

Figure 6. Overall survival in mismatched transplant

Discussion

Management of chronic GVHD remains a major challenge, and has become a significant health problem in HSCT survivors with the increasing use of mobilized peripheral blood stem cells (PBSC). Furthermore, calcineurin inhibitors (CNI)-based immune suppression regimens are associated with considerable toxicity.[2]

Regarding hepatic toxicity of the GVHD prophylaxis regimen, cyclosporine inhibits canalicular bile transport and commonly causes elevation of serum

aminotransferase enzymes. Tacrolimus less commonly causes cholestasis, except in the setting of toxic blood levels. An extreme rise in ALT is now mostly due to a non-infective cause such as zone3 hepatocyte necrosis in SOS, hypoxic hepatitis, drug-induced liver injury, or a hepatitic presentation of GVHD.[5] Calcineurin inhibitors are also known nephrotoxic agents, most commonly secondary to their potent vasoconstricting properties and ability to cause endothelial injury. Despite this, the majority of studies fail to show a significant association between cyclosporine blood levels and development of acute kidney injury (AKI).[6]

In our study, patients receiving conventional prophylaxis had statistically significant higher rates of hepatic and renal toxicity compared to patients receiving post-transplant cyclophosphamide (PTCy). However, cyclophosphamide resulted in higher rate of hemorrhagic cystitis which was not statistically significant due to low number of patients. In another report, and despite high cyclophosphamide doses, only 1 patient receiving PTCy required significant intervention for higher grade hemorrhagic cystitis.[7] Although small numbers do not allow conclusive analysis, this different toxicity profile could be considered in patient selection for the appropriate GVHD prophylaxis especially in those with previous comorbidities.

Earlier reports have suggested PTCy GVHD prophylaxis results in similar rates of acute GVHD as CNI-based prophylaxis in patients receiving myeloablative conditioning.[8] Results of our study suggest that the rate of acute GVHD was not significantly different in patients receiving PTCy compared to patients who received conventional GVHD prophylaxis. In fact, the rate of acute GVHD was numerically higher in patients receiving post-transplant CY (20.5% vs. 11.1%) which could be explained by the higher percent of haplo-transplants in PTCy group.

Also differing from the earlier reports, in our study we did not see a reduction in the rate of chronic GVHD with the PTCy regimen which is probably due to the same cause as in rate of acute GVHD. Additionally, the predominant use of BM grafts in the earlier reports may account for the lower rates of chronic GVHD.[9-12] The use of PTCy alone, without the use of any additional IS drugs, for GVHD prophylaxis in the setting of matched sibling donor or 10/10 HLA matched unrelated donor was initially reported by Luznik[8] and, subsequently, in a multicenter study,[13] both demonstrating the efficacy of this strategy. Importantly, in these studies, BM was the sole stem cell source. Notably, these findings have not been demonstrated with PBSC.

Alousi [14] published the results of a phase II clinical trial using PBSC and RIC regimen indicating an excess of acute and chronic GVHD and NRM and therefore recommended the use of the standard GVHD prophylaxis in HSCT following RIC regimen in combination with PBSC grafts. The same findings determined the early closure of a different prospective phase 2 trial after four cases of severe acute GVHD, and related toxicity was reported on the first five patients enrolled. **[15]**

PB was the main graft source used in our patients, making it important to determine if this source can be employed with the PTCy strategy. Our data showed a trend toward a higher rate of both acute and chronic GVHD after PTCy GVHD prophylaxis; however, this did not reach statistical significance in this relatively small study. These results suggest caution should be used when using PB grafts with PTCy.

In the original report by luznik and colleagues [8] using PTCy, reactivation of CMV occurred in 32% of patients, and there was only one documented case of CMV disease and no CMV-associated mortality. In our patients, the rate of CMV reactivation was similar between both groups. This supports the data from other studies in which the incidence of CMV reactivation did not differ between PTCy and tacrolimus/MTX GVHD prophylaxis.**[16]**

In our patients, neither DFS nor OS showed statistically significant difference between both groups. This conclusion is consistent with Bashey et al.'s [15] study of 53 patients receiving PTCy in comparison with patients treated with matched related or unrelated donor transplants receiving standard GVHD prophylaxis. Similarly, a matched controlled analysis of 2 groups receiving either PTCy or tacrolimus and methotrexate as GVHD prophylaxis after matched related and unrelated donor reduced-intensity transplants showed the same rates of DFS and OS.**[12]**

However, in our study, there was a strong trend toward better OS which didn't reach significance mostly due to small patients' number. Increasing degrees of HLA mismatch between donor and recipient have been repeatedly associated with greater toxicity and inferior survival after allogeneic BMT.**[16-18]** However, the present study suggests that in HLA-mismatched transplants, patients receiving PTCy may have better OS than patients receiving standard immunosuppression (P=0.074). Other reports have also suggested that HLA disparity does not worsen overall outcome in this subset of patients.**[19]**

Conclusion:

We conclude from this study that using PTCy for GVHD prophylaxis is safe and effective in patients undergoing allogeneic stem cell transplantation, and is a valid option with a different toxicity profile compared with CNI regimens. Further research is needed to clarify the validity of using PTCy when utilizing PBSC as the main graft source in allogeneic stem cell recipients.

Ethical and consent:

The authors would like to thank you for your comment, and we confirm that the study was conducted in one of the biggest governmental hospital and uder a full governmental supervion, Also we confirm that a prior permission was obtained from specialized institutional ethics committee based on a written consents from the patients prior to the study as needed, so we would like to inform you that we followed all the regulatory guidelines , thank you again

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