Biol Blood Marrow Transplant 25 (2019) 2375–2382

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Outcome of Fludarabine-Based Conditioning in High-Risk Aplastic Anemia Patients Undergoing Matched Related Donor Transplantation: A Single-Center Study from Pakistan

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Article history:
Received 6 June 2019
Accepted 23 July 2019

Key Words:
Aplastic anemia
Fludarabine
Stem cell transplantation

A B S T R A C T

Despite excellent transplant outcomes of aplastic anemia (AA) in developed countries, management in developing countries is challenging because of delay in the diagnosis, use of family donors for transfusions, and higher infection risk pretransplant. These factors can lead to allo-immunization, increased risk of graft failure, graft-versus-host disease (GVHD), and transplant-related mortality, leading to unfavorable outcomes. Conventional cyclophosphamide (Cy) and antithymocyte globulin (ATG) are associated with inferior overall survival in such high-risk patients. We conducted single-center retrospective analysis of high-risk AA patients (N = 147) enrolled consecutively and undergoing matched related donor transplant from March 2002 through October 2018. We included high-risk AA patients receiving fludarabine (Flu)-based conditioning. Median patient age was 20 years (range, 3 to 52). The median time from diagnosis to transplant was 11 months (range, 3 to 63). High-risk features included age $\geq$ 20 years in 55.8% of patients (n = 82), disease duration more than 3 months in 95 % (n = 140), RBC concentrates transfusions $>$ 20 in 79.6% (n = 117), random donor platelet transfusion $>$ 50 in 64.6% of patients (n = 95), and second hematopoietic stem cell transplant (HSCT) in 7.4% (11). We divided patients into 2 groups based on different conditioning regimens. Flu group 1 (Flu1) received Flu 120 to 150 mg/m^2, Cy 120 to 200 mg/kg, and ATG 20 mg/kg, and Flu group 2 (Flu2) was given Flu 150 mg/m^2, Cy 300 mg/m^2, and ATG 20 mg/kg. Bone marrow stem cells were used as graft source in 97% of patients (n = 144) (alone in 52% and with peripheral blood stem cells in 45%). Cyclosporine alone was used for GVHD prophylaxis in 75% (n = 110) and cyclosporine plus methotrexate in 25% (n = 37). Median total nucleated cell dose was 5×10^8/kg. Median days for neutrophil engraftment was 13 (range, 10 to 20) and platelet engraftment 20 (range, 14 to 43). Day 100 mortality was 7.5% (n = 11). Sustained successful engraftment was achieved in 87.8% of patients (n = 129). Most graft failures (40%) occurred in Flu2 conditioning (P = .000) and in patients with $>$2 risk factors (P = .000). Overall incidence of acute and chronic GVHD was 11.6% (n = 17) and 12.9% (n = 19), respectively, in Flu1 and Flu2 groups. Overall survival (OS), disease-free survival (DFS), and GVHD-free relapse-free survival (GRFS) was 83.7%, 78.2%, and 70.7%, respectively. A trend toward improved OS was observed in patients receiving Flu1 conditioning but was statistically nonsignificant (P = .256), whereas DFS and GRFS were significantly better in Flu1 versus Flu2 (P = .004 and .001, respectively). When stratified per number of risk factors (age $>$ 20, RBC concentrate $>$ 20 or platelet $>$ 50 random, duration $>$ 3 months, previous HSCT), OS and DFS decreased significantly with increasing number of risk factors (P = .000 and .001, respectively). Patients are able to tolerate Flu-based conditioning well with lower rates of rejection and excellent long-term survival in high-risk AA patients. Cyclosporine alone as GVHD prophylaxis and marrow source stem cells as graft source are preferable options. Use of Flu plus low-dose Cy conditioning is associated with inferior survival outcomes. A randomized trial of Flu-based versus conventional Cy-containing conditioning would be

Financial disclosure: See Acknowledgments on page 2382.
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https://doi.org/10.1016/j.bbmt.2019.07.029

helpful in establishing a standard of care conditioning regimen in high-risk AA patients.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has revolutionized the outcome of aplastic anemia (AA) and remains the treatment of choice for HLA matched younger patients with severe and very severe AA [1]. Advances in supportive care and better patient selection have significantly improved transplant results over the last 2 decades, with overall survival (OS) close to 90% in developed countries [2-4].

In developing countries like Pakistan, treatment of AA continues to be a significant challenge because of delay in diagnosis, lack of high-quality blood bank services, financial constraints, and limited availability of transplant. Because of delay in accurate diagnosis, patients have multiple episodes of infections before HSCT. They receive either whole blood transfusions or blood from first-degree relatives and get random donor platelet transfusions. These factors contribute to high risk of alloimmunization, graft failure, infectious complications, and increased mortality as documented in the literature [5-7]. Traditionally, cyclophosphamide (Cy) and antithymocyte globulin (ATG) are the standard conditioning regimen, and cyclosporine with or without methotrexate (MTX) is used for graft-versus-host disease (GVHD) prophylaxis.

Fludarabine (Flu)-based conditioning has been used in various studies to reduce the risk of graft rejection and improve OS in high-risk AA patients; however, most of these studies are small, used different regimens, and yielded variable outcomes [8-10]. We present our single-center experience of 147 consecutive high-risk AA patients treated with a Flu-based conditioning regimen. To our knowledge, this is largest single-center study of high-risk AA patients undergoing matched related donor transplant using Flu-based conditioning.

METHODS

This is a single-center retrospective analysis of 147 high-risk AA patients who underwent matched related donor HSCT at the Armed Forces Bone Marrow Transplant Centre/National Institute of Bone Marrow Transplant from March 2002 to October 2018. All recipients received stem cells from 6 antigen matched sibling or family donors (144 siblings, 2 parents, 1 nephew), determined by DNA-based low/intermediate resolution typing for HLA class I and class II antigens. AA was defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate or marrow fibrosis, and severity was graded as per Camitta et al. [11] and Bacigalupo et al. criteria [12]. Risk factors were defined according to European Society for Blood and Marrow Transplantation (EBMT) registry data risk stratification and institutional criteria.

We considered patients meeting any of the following criteria as high risk: age $\geq$ 20 years, time from diagnosis to transplant $>$ 3 months, heavily transfused $>$ 20 RBC transfusions or $>$50 random platelets, and failed previous HSCT. Inclusion criteria were high-risk AA patients, age $>$ 2 years, and informed written consent for recipient and donor. Exclusion criteria included major psychiatric illnesses, pregnancy, high hematopoietic cell transplant–specific comorbidity index, and patients lost to follow-up. The institute's Ethical Review Board and Research Department reviewed and approved the study.

Transplant Procedure and Supportive Care

Following routine standards and precautions of our institution for allogenic transplant, we admitted patients to isolation rooms with laminar airflow and HEPA filters. We implemented the standard protocol for neutropenic patients as per the institute's infection prevention guidelines. Methicillin-resistant Staphylococcus aureus screening and stool sampling for vancomycin-resistant enterococci and carbapenamase-resistant enterococci were performed at the time of admission. We performed cytomegalovirus (CMV) monitoring for reactivation on weekly basis starting at day +30 until day +100 using quantitative CMV PCR testing. All patients received antiviral, antifungal, and *Pneumocystis jirovecii*prophylaxis.

Doses and schedule of Flu-based conditioning varied in our study. We used the following conditioning protocols in descending order of frequency:

- Flu 120 mg/m^2/Cy 120 mg/kg/ATG 20 mg/kg
- Flu 150 mg/m^2/Cy 300 mg/m^2/ATG 20 mg/kg
- Flu 150 mg/m^2/Cy 120 mg/kg /ATG 20 mg/kg
- Flu 120 mg/m^2/Cy 160 mg/kg /ATG 20 mg/kg
- Flu 120 mg/m^2/Cy 200 mg/kg /ATG 20 mg/kg

We used ATG Fresenius (Neovii-Biotech, Graefelfing, Germany) in our patients, which contains the Jurkat cell line origin. We used ATG at a higher dose of 20 mg/kg to reduce the risk of primary graft failure and GVHD as a result of heavy pretransplant alloimmunization of the study cohort. In our study 11 patients (7.4%) received a second HSCT. Of 11 patients, 8 patients had received Cy 200 mg/kg and ATG Fresenius 20 mg/kg as first conditioning regimen; 2 patients received Flu 150 mg/m^2, Cy 300 mg/m^2, and ATG 20 mg/kg; and 1 patient received Flu 120 mg/m^2, Cy 120 mg/m^2, and ATG 20 mg/kg. For purposes of data analysis we labeled patients receiving low-dose Cy (Flu 150/Cy 300 mg/m^2/ATG 20) as Flu group 2 (Flu2) and those receiving all other protocols using intermediate to high dose Cy as Flu group 1 (Flu1). Graft sources included bone marrow harvest (BMH), combined BMH and peripheral blood stem cell harvest, and peripheral blood stem cells alone. We based selection of graft source on weight disparity between donor and recipient, major ABO mismatch, availability, and donor choice.

GVHD prophylaxis included cyclosporine alone 3 mg/kg i.v. twice daily that was later transitioned to oral cyclosporine with target trough levels of 200 to 300 ng/mL, continued until 12 months, and gradually tapered off by 10% every week with close observation. Other GVHD prophylaxis used included cyclosporine plus short-course MTX (10 mg/m^2 day +1 and 8 mg/m^2 days +4 and +7). We performed whole blood chimerism for post-transplant monitoring on days +28, +100, and +180 or as needed per clinical indication (eg, drop in blood counts). Ganciclovir and valgancyclovir were used for preemptive CMV treatment if $>$2000 copies/mL were detected and were continued until 2 consecutive PCR results were negative.

All patients received irradiated and leukodepleted blood products. We defined neutrophil engraftment as the first of 3 consecutive days with absolute neutrophil count $> .5 \times 10^9$/L. We defined platelet engraftment as platelet count $> 20 \times 10^9$/L that was unsupported for 7 days. We defined primary graft failure as failure to achieve neutrophil engraftment by day +28. We defined secondary graft failure as persistent neutropenia (absolute neutrophil count $< .5 \times 10^9$/L) with loss of donor chimerism after initial engraftment with hypocellular bone marrow. We defined acute GVHD as clinical symptoms and signs of skin rash, loose stools, and jaundice early post-transplant graded according to Glucksberg criteria. We defined chronic GVHD clinically based on the chronic GVHD per cases presenting after day +100 and divided into limited and extensive as per National Institutes of Health–defined criteria. Those with symptoms and signs of both acute and chronic GVHD were labeled as having overlap syndrome. Based on clinical indication we used standard dose steroids, cyclosporine mycophenolate mofetil, mesenchymal stem cells, rituximab, ibrutinib, and thalidomide for the treatment of GVHD.

Statistical Analysis

We calculated OS by including all patients who were alive at the time of last follow up. We calculated disease-free survival (DFS) as survival in the absence of rejection. GVHD-free relapse-free survival (GRFS) included all patients who were alive and free of GVHD and disease relapse on the last evaluation. A chi-square test was used for categorical variables, whereas continuous variables were compared using either Student's *t*-test or Mann-Whitney U test. We used univariate and multivariate Cox regression analysis to determine significance of different variables and their effect on survival. We used the Kaplan-Meier method to determine the probability of OS, DFS, and GRFS. We used the log rank test to assess differences in OS and DFS between groups. We considered $P \leq .05$ to be statistically significant. We used SPSS (IBM Corp., Armonk, N.Y., USA) version 23.0 to complete our statistical analysis.

RESULTS

Patient Characteristics

Based on the defined inclusion criteria, we enrolled 147 consecutive patients meeting the criteria for high-risk AA. We summarize patients' characteristics in Table 1. There were 116

Table 1
Demographic Characteristics of the Study Population (N = 147)

Characteristics	Value
Age, yr	20 (3-52)
Age groups	
>2-10 yr	12 (8.2)
11-20 yr	63 (42.9)
21-30 yr	57 (38.8)
31-40 yr	10 (6.8)
>40 yr	5 (3.4)
Recipient, male-to-female	3.7:1
Time from diagnosis to transplant, days	330 (87-1890)
PNH clone	8 (5.4)
Failed previous HSCT	11 (7.4)
Serum ferritin, ng/mL	1328 (63-4310)
RBC concentrate transfused	35 (10-110)
Platelets transfused	60 (6-150)
Gender mismatch donor–recipient (female to male)	47 (32)
Major ABO mismatch	33 (22)
Disease severity	
NSAA	7 (4.8)
SAA	94 (64)
VSAA	46 (31.2)
Donor age, yr	21 (2-50)
Donor, male-to-female	1.3:1

Values are median (range) or n (%). PNH indicates paroxysmal nocturnal hemoglobinuria; NSAA, nonsevere aplastic anemia; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia.

male and 31 female patients (male-to-female ratio, 3.7:1), and median age was 20 years (range, 3 to 52).

The median time from diagnosis to transplant was 11 months (range, 3 to 63). Paroxysmal nocturnal hemoglobinuria clone was present in 5.4% of patients, but no patient had clinical evidence of hemolysis. High-risk features of the study population included age ≥ 20 years in 55.8% of patients (n = 82), disease duration more than 3 months in 95% (n = 140), RBC concentrate transfusion > 20 in 79.6% (n = 117), random donor platelet transfusion > 50 in 64.6% (n = 95), and 7.4% (11). In our study cohort 5.4% of patients (n = 8) received pretransplant immunosuppression. Two of these 8 patients were diagnosed with nonsevere AA and were given ATG plus cyclosporine (both patients were given rabbit ATG because of the unavailability of horse ATG), whereas 6 patients were diagnosed with severe AA. Of these 6 patients, primary physicians gave 4 patients cyclosporine and 2 patients prednisolone before referral to our center. None of the patients responded to immunosuppression.

Transplant Characteristics and Engraftment

Conditioning regimens have differed since 2002. We summarized GVHD prophylaxis and other transplant characteristics in Table 2.

Transplant Outcomes and Complications

Our study found that 129 patients (87.8%) achieved successful sustained engraftment. We summarize this information in Table 3. Primary graft failure occurred in 8 patients (5.4%) and secondary graft failure in 10 (6.8%). Most graft failures occurred in patients in the Flu2 group (P = .000) and with >2 risk factors (P = .000). Most commonly, we used cyclosporine alone (75%) for GVHD prophylaxis. The cumulative incidence of acute GVHD was 11.6%, with an incidence of grades II to IV

Table 2
Transplant Characteristics and Engraftment of Study Population

Transplant Characteristics	Value
Conditioning protocols used	
Flu 120 mg/m^2, Cy 120 mg/kg, ATG 20 mg/kg	90 (61)
Flu 120 mg/m^2, Cy 300 mg/m^2, ATG 20 mg/kg	25 (17)
Flu 150 mg/m^2, Cy 120 mg/kg, ATG 20 mg/kg	17 (12)
Flu 120 mg/m^2, Cy 160 mg/kg, ATG 20 mg/kg	12 (8)
Flu 120 mg/m^2, Cy 200 mg/kg, ATG 20 mg/kg	3 (2)
Graft source	
BMH	78 (52)
G-CSF–primed BMH + PBSCs	65 (45)
PBSC harvest	4 (3)
GVHD prophylaxis used	
Cyclosporine	110 (75)
Cyclosporine plus MTX	37 (25)
Nucleated cell dose, $\times 10^8$/kg	5 (2.1-10.79)
CD34 dose, $\times 10^6$/kg	5.8 (1.53-11.7)
Neutrophil engraftment, days	13 (10-20)
Platelet engraftment, days	20 (14-43)

Values are median (range) or n (%). G-CSF indicates granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell.

acute GVHD of 9.8% and of grade IV acute GVHD of 2.6%. Nineteen patients (12.9%) developed chronic GVHD, and limited chronic GVHD was present in 13 (8.8%) and extensive chronic GVHD in 6 (4.1%). There was no effect of type of GVHD prophylaxis used (cyclosporine alone or cyclosporine plus MTX) on frequency and severity of acute or chronic GVHD (P = .949). Type of GVHD prophylaxis had no statistically significant effect on outcome. OS in those who received cyclosporine alone and cyclosporine plus MTX was 86.9% and 78.3%, respectively (P = .163), whereas DFS was 82.2% in those who received cyclosporine alone and 72.9% in those who received cyclosporine plus MTX (P = .165). Survival free of acute and chronic GVHD and relapse (GRFS) was 76.6% in those who received cyclosporine and 59.5% in those who received cyclosporine plus MTX (P = .03) (Figure 1). OS and DFS was 87.5% and 78.3% in patients receiving prior immunosuppressive therapy. Use of prior immunosuppressive therapy was not associated with adverse outcomes in our study.

Hemorrhagic cystitis developed in 12 patients (8.2%); of these, 4 patients (2.7%) had BK virus reactivation detected by PCR. Patients responded to supportive therapy; 1 patient required cidofovir and 1 patient required urologic intervention with roller ball diathermy. Erythrocytosis was an unusual complication seen in 10 patients (6.8%) at a median of 12 months. Post-transplant, these patients were negative for *JAK-2*

Table 3
Transplant Complications

Complication	No. of Cases (%)
Graft failure	
Primary	8 (5.4)
Secondary	10 (6.8)
Acute GVHD grades I-IV	17 (11.6)
Chronic GVHD	19 (12.9)
Thrombotic microangiopathy	6 (4)
CMV reactivation (47 assessable patients)	13 (27.6)
Serum sickness	4 (2.7)
Erythrocytosis	10 (6.8)
Hemorrhagic cystitis	12 (8.2)

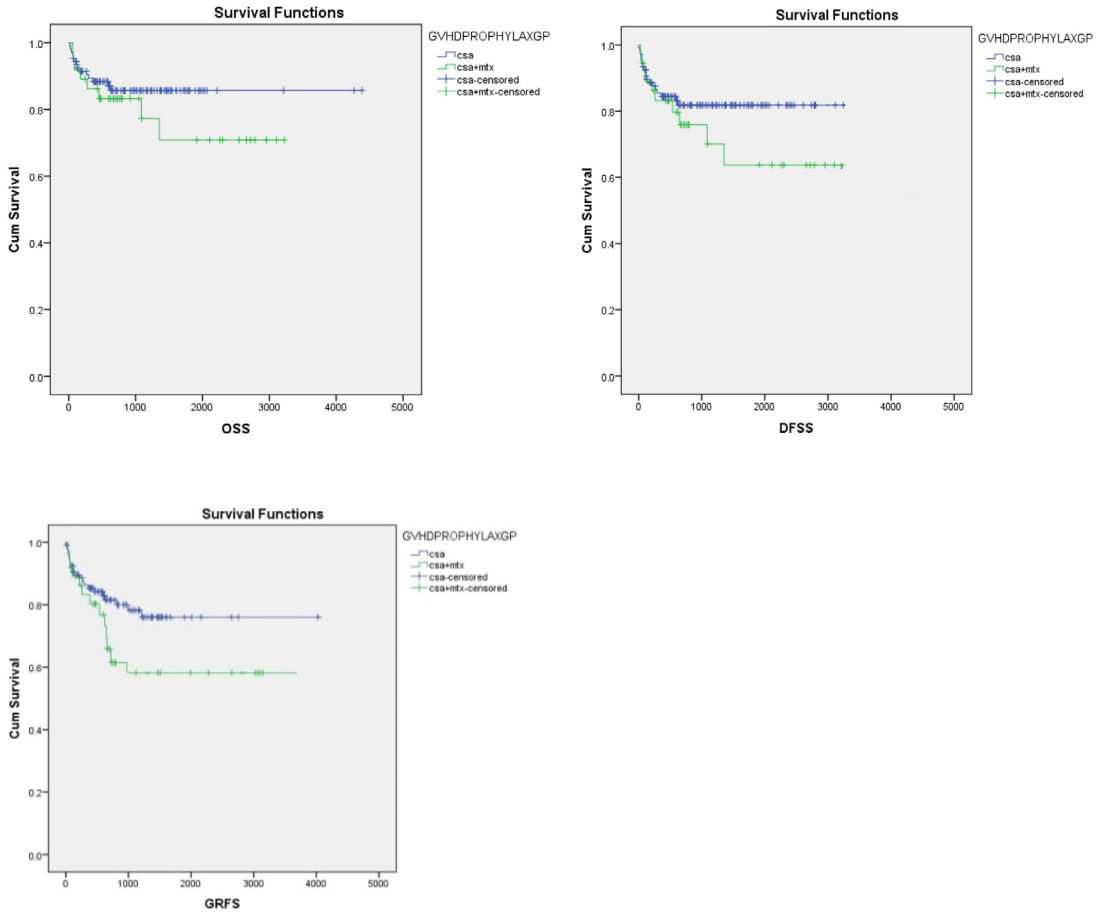

Figure 1. Effect of GVHD prophylaxis on OS and DFS. (A) OS in cyclosporine alone and cyclosporine plus MTX was 86.9% and 78.3%, respectively ($P = .163$). (B) DFS was 82.2% in cyclosporine alone and 72.9% in cyclosporine plus MTX ($P = .165$). (C) GRFS was 76.6% in cyclosporine and 59.5% in cyclosporine plus MTX and was statistically significant ($P = .03$).

mutation, and Erythropoietin levels were within normal limits. For the purpose of this study, we defined erythrocytosis as hemoglobin more than 17 g/dL in males and 16 g/dL in females or hematocrit more than 52% in males and 48% in females with an absence of clonal markers and other causes of secondary erythrocytosis. Median age at development of erythrocytosis was 21.2 years (range, 16 to 28.5), and median time from transplant to erythrocytosis was 33 months (range, 18 to 51). All patients who developed erythrocytosis were males. We performed therapeutic phlebotomy in all cases to keep hematocrit less than 50%. None of the patients developed any thrombotic complication.

Transplant-associated thrombotic microangiopathy was seen in 4 patients (4%), and 4 of these patients died. CMV reactivation was seen in 27.6% of assessable patients (n = 47). Use of steroids (>3 days) was associated with statistically significant risk of CMV reactivation ($P = .01$). Serum sickness occurred in 4 patients (2.7%) at a median of 24 days (range, 17 to 34) after first ATG exposure. Reactivation of tuberculosis occurred in 8 patients (5.4%).

Survival

Median follow-up of the study group was 32 months (range, 6 to 146). Eleven patients died within 100 days of transplant with a 100-day mortality of 7.5%. There was no difference in day 100 mortality as per choice of Flu-based conditioning ($P = .410$). OS of the study cohort was 83.7%, DFS was 78.2%, and GRFS was 70.7%. When patients were stratified into 2 groups, the Flu1 group versus the Flu2 group, OS was 86.1% versus 72% ($P = .256$), DFS 84% versus 48% ($P = .004$), and GRFS 77.9% versus 36%, respectively ($P = .001$) (Figure 2). On univariate analysis of age subgroups (as mentioned in Table 1) with survival, difference in OS was statistically significant ($P = .049$); the P value for DFS was .313 (Figure 3).

Because of improvement in supportive care and use of Flu1 conditioning, OS and DFS were significantly better in patients transplanted after 2014. All these received Flu1 conditioning (Figure 4). OS and DFS were superior in patients receiving granulocyte colony-stimulating factor–primed BMH (92.9% and 87.6%, respectively) as compared with patients receiving unprimed BMH (81.3% and 73.4%, respectively) and in patients receiving peripheral blood stem cells (72.1% and 66.7%, respectively); however, the differences in OS and DFS were not significant statistically.

Overall, 24 patients died after transplant, with the most common cause of death being graft failure (6.8%, n = 10) (Table 3). Three patients died of primary graft failure and 7 patients died of secondary graft failure. Infectious mortality was seen in 2.7% of patients (n = 4), acute GVHD in .7% (n = 1), chronic GVHD in 2% (n = 3), bleeding complications in .7% (n = 1), thrombotic microangiopathy in 2.7% (n = 4), and renal failure in .7% (n = 1).

Eighty-seven patients received HSCT before 2014: 62 (71.2%) in the Flu1 group and 25 (28.8%) in the Flu2 group. We summarize a univariate analysis of different variables as per

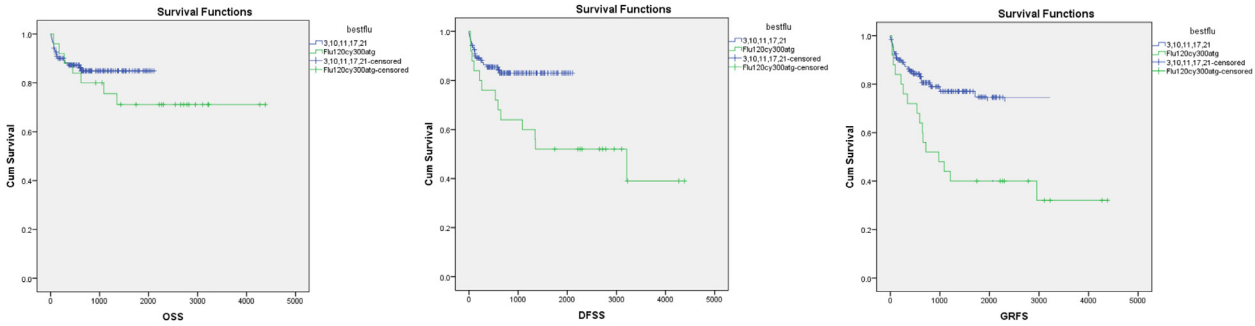

Figure 2. OS and DFS as per conditioning regimen in Flu1 and Flu2 groups. (A) OS was 86.1% in Flu1 and 72% in Flu2 groups ($P = .256$). (B) DFS was 84% in Flu1 and 48% in Flu2 groups ($P = .004$). (C) GRFS was 77.9% in Flu1 and 36% in Flu2 groups ($P = .001$).

conditioning regimen in patients receiving HSCT before 2014 in Table 4. Patients receiving Flu2 had significantly higher incidence of secondary graft failure and lower DFS and GRFS. On multivariate analysis disease duration > 3 months, RBC concentrate transfusions > 20, platelet transfusions > 50, previous HSCT, and transplant before 2014 had statistically significant effect on OS ($P = .04$, .02, .01, .001, and .001, respectively), whereas age at transplant had a statistically nonsignificant effect on OS ($P = .06$) (Figure 5). Data were split in 3 quartiles per time of HSCT (Q1, 2002 to 2006; Q2, 2007 to 2013; Q3, 2014 to 2018), and all above-mentioned factors remained significant in multivariate analysis statistically except age

DISCUSSION

Prevalence of AA is higher in Asian countries as compared with Western populations [13]. Management of AA in developing countries poses unique challenges including increased risk of alloimmunization, graft failure, organ toxicity, and severe life-threatening infections, with resultant inferior OS and DFS.

Two important sources for definition of high-risk AA include EBMT data (1) and a study by George et al. [14]. In EBMT registry data patients with 1, 2, and 3 to 4 risk factors had an OS of 89%, 78%, and 64%, respectively. However, these EBMT data did not include previous immunosuppressive therapy or transplant and no RBC concentrate or platelet transfusions, which are recognized risk factors for graft rejection in different studies [5,8]. Considering the literature from other sources and our institutional experience (n = 1450 AA patients), we used a unique Armed Forces Bone Marrow Transplant Centre definition for high-risk AA in this study.

Over the years Flu has emerged as a powerful immunosuppressive agent with manageable systemic toxicities when used in HSCT [9]. The drug is widely available, has low acquisition cost, has potent immunosuppression and lymphoablative properties, and is well tolerated. Studies have shown that Flu-based conditioning regimens in AA allow engraftment in heavily presensitized individuals with acceptable toxicity and favorable outcome [10,15]. When combined with Cy it works synergistically by inhibition of alkylation induced DNA repair [16].

Our center has a large cohort of 1450 AA patients, and our institutional data show, at the time of transplant, 81% patients had 1 or more high-risk feature. We gave a Flu-based conditioning regimen to these high-risk patients with a goal of reduced conditioning toxicity, reduced risk of graft failure, and improved OS and DFS. Following are some of the unique factors in our AA cohort. Median age in our study group was 20 years, and median age of our entire AA cohort of register patients in the database was 21 years. Our cohort of Pakistani patients was younger compared with published Western data and reflects disease presentation at a younger age in this part of the world rather than a selection bias in the study. Moreover, median age was comparable with studies by Al-Zahrani et al. [17], Mahmoud et al. [18] Kumar et al. [15], and George et al. [14] from the same region [18]. The male-to-female ratio was 3.7:1 and is a reflection of social bias to provide costly

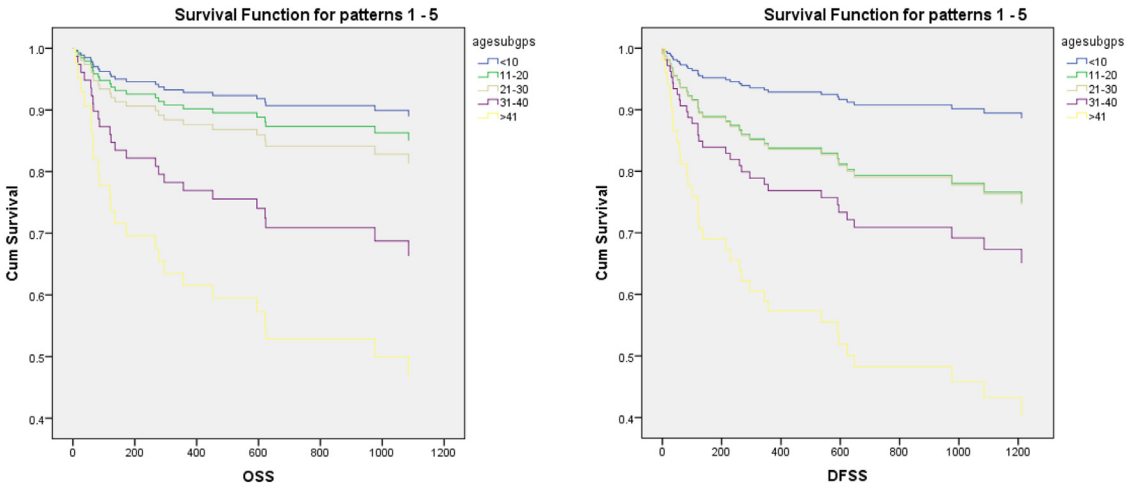

Figure 3. Effect of age groups on OS and DFS. (A) OS was 91.7%, 87.3%, 84.2%, 70%, and 40% per age groups. (B) DFS was 91.7%, 79.4%, 78.9%, 70% and 40% per age groups.

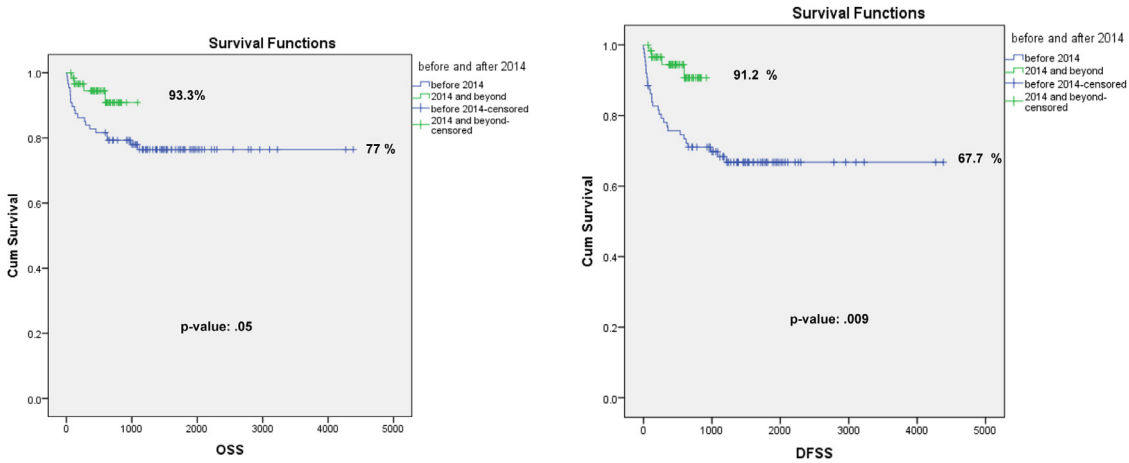

Figure 4. OS and DFS as per time of HSCT. (A) OS was 93.3% and 77% (hazard ratio, 2.82; 95% confidence interval, .095 to 8.3; $P = .05$). (B) DFS was 91.2% and 67.7% (hazard ratio, .244; 95% confidence interval, .085 to .705; $P = .009$).

care preferentially to males. Median time from diagnosis to transplant was 11 months (range, 3 to 63), which was much longer compared with North American and Western data [1] and regional data using Flu-based conditioning [14,17]. This prolonged median time from diagnosis to transplant is likely due to a number of factors, including delay in diagnosis, delay in referral to transplant center, financial constraints, and long transplant waiting lists. Consequently, most of these patients with severe and very severe AA receive a large number of RBC concentrate and platelet transfusions before transplant and whole blood transfusions (transfusions from close family members and even from potential donors). Moreover, random donor platelets without leukodepletion is the most common method of platelet transfusion because of the very high cost of single-donor apheresis and lack of prestorage leukodepletion facilities. All these factors lead to a high rate of alloimmunization in our AA patients awaiting bone marrow transplant, which has a direct correlation with graft failure and poor transplant outcomes.

We tabulate conditioning regimens used in our center over the last 16 years in Table 2. In our study we observed sustained engraftment in 87.8% of the study cohort and in 93.9% of patients receiving Flu1 conditioning. Primary graft failure occurred in 5.4% of patients (n = 8) and secondary graft failure in 6.8% (n = 10). When compared by type of Flu-based conditioning (Flu1 versus Flu2), we saw 9 of 10 cases of secondary graft failure in Flu2 patients. We assume that low-dose Cy is insufficient to achieve stable sustained engraftment in high-risk patients. This was the reason we did not use low-dose Cy conditioning after 2013 and switched to Flu1 in high-risk cases. When compared with available literature, engraftment was seen in 97.5% of an Indian cohort, but patients had shorter median time from diagnosis to transplant, had received lesser transfusions, and only 62.8% of their patients were labeled as high risk in that study [14].

Similarly, in a Chinese study that included heavily pretransfused patients receiving Flu-based conditioning with high-dose Cy, researchers saw engraftment in 92.7% of patients, but engraftment was compounded by very high rates of acute and chronic GVHD and regimen-related toxicities [19]. A dose finding study of Cy in unrelated patients undergoing bone marrow transplant in AA documented that reducing the dose of Cy increased the risk of graft failure [20]. BMH was the most commonly used source of graft and had superior OS as compared with peripheral blood stem cells, a finding repeatedly reported in the literature of AA patients receiving transplant.

There was no difference in frequency of acute or chronic GVHD in patients receiving cyclosporine alone or cyclosporine plus MTX ($P = .949$) after adjusting for covariates. This observation was similar to the results of Srinivasan et al. [8]. On the contrary, OS, DFS, and GRFS were better in the cyclosporine alone group. This may be due to increased mucosal injury caused by MTX, leading to increased expression of damage-associated molecular patterns and cytokines, which are a

Table 4
Univariate Analysis of Study Characteristics and Transplant Outcome Per Conditioning Protocol in Patients Receiving HSCT before 2014

Variable	Flu1 Group (n = 62)	Flu2 Group (n = 25)	*P*
Median age, yr	21	18	.586
Median disease duration, mo	10	8.4	.756
Mean serum ferritin	1710	2230	.65
Median no. of RBC concentrate transfusions	32.5	27.5	.281
Median no. of platelet transfusions	62.5	50	.399
OS, %	79	72	0.33
DFS, %	75.8	48	.013
GRFS, %	71	36	.003
Primary graft failure, n (%)	6 (9.6)	1 (4)	—
Secondary graft failure, n (%)	1 (1.6)	9 (36)	.000
Median neutrophil engraftment (range)	13 (10-16)	14 (10-19)	.581
Median platelet engraftment (range)	20 (14-41)	19 (15-40)	.291

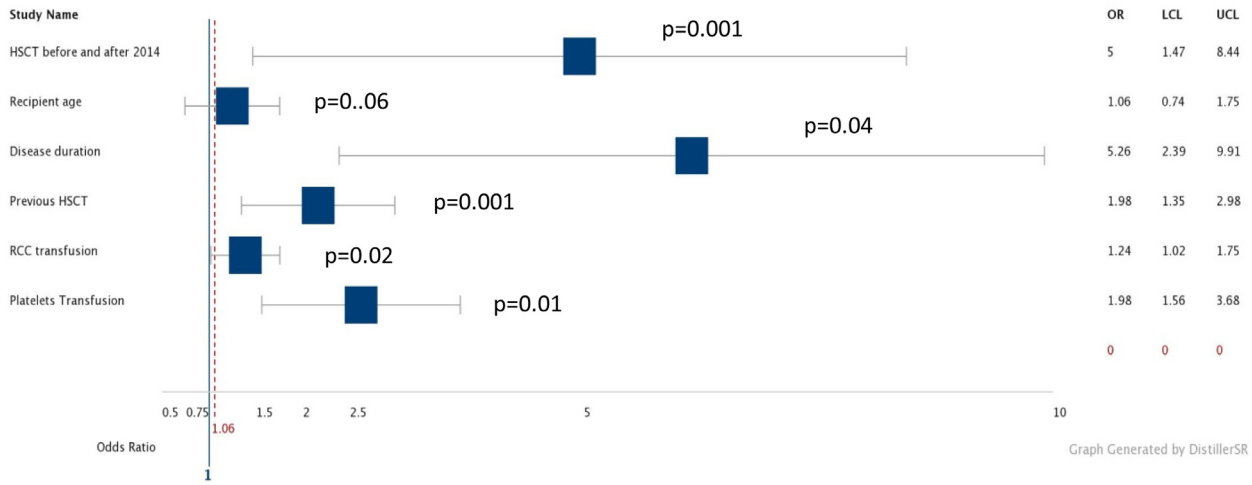

Figure 5. Multivariate analysis of factors affecting OS in high-risk AA patients receiving HSCT with Flu-based conditioning. A multiple regression analysis was run to predict OS from time of HSCT (before and after 2014), recipient age, disease duration, previous HSCT, and number of RBC concentrate and platelet transfusions.

known trigger for GVHD. However, we are unsure of the exact explanation of this finding.

Despite a high-risk cohort, the frequency of acute and chronic GVHD was low and translated into high GRFS as described above. We compare conditioning regimens, GVHD, engraftment days, and OS in Table 2.

Our study is the only study to enroll high-risk patients and achieved a good balance of low toxicity, low rates of GVHD, and better OS and DFS (Table 5). When analyzed by type of conditioning regimen, the Flu1 group achieved higher OS, DFS, and GRFS than the Flu2 group because there was a high frequency of secondary graft failure in patients receiving low-dose Cy, a finding also documented in other studies [20].

Since the establishment of our center in 2001 improvements in supportive care and refinements in transplant protocol survival rates have improved. Consequently, OS, DFS, and GRFS rates before 2014 were 77%, 67.8%, and 60.9% and after 2014 were 93%, 89%, and 81%, respectively. Another factor contributing to better OS was choice of conditioning regimen, and 100% of patients received Flu1 conditioning after 2014.

Because survival in our study cohort was inversely affected by the number of risk factors present, we performed multiple regression analyses to predict the effect of these variables on OS and found that all variables had statistically significant effects on outcome except age, which had a statistically non-significant effect on OS. Whether Flu-based conditioning mitigated poor prognostic effect of age in our study cohort needs to be studied further, and large-scale studies are required in this regard.

This retrospective analysis further supports the fact that Flu-based conditioning provides favorable outcomes in high-risk AA patients undergoing matched related transplant. This study is unique in that it included only high-risk patients and provided comparison of different Flu-based conditioning. This study provides a basis to consider randomized trials of Cy/ATG versus Flu/Cy/ATG in low- and high-risk AA patients to establish a gold standard regimen in these subgroups of patients with the aim to further improve outcome in this otherwise fatal disorder.

In conclusion, our study found that high-risk AA patients were able to better tolerate Flu-based conditioning with lower rates of rejection and excellent long-term survival. Cyclosporine alone as GVHD prophylaxis and BMH as graft source are preferable options. Use of Flu plus low-dose Cy conditioning is associated with inferior survival. A randomized trial of Flu-based versus conventional Cy conditioning would be helpful in

Table 5
Comparison of Studies Using Flu-Based Conditioning in AA Patients

	Qammar et al	Srinivasan et al [8]	Chan et al [21]	George et al [14]	L-zahrani et al [17]
Number	147	26	5	121	38
Age years, median (range)	20 (3-52)	30 (11-65)	0.7-11.5	22 (2-54)	20 (14-36)
Conditioning regimen	1. Flu120-150mg/m^2:Cy 120-200 mg/kg:ATG 20 mg/kg 2. Flu 120mg/m^2:Cy 300 mg/m^2:ATG 20 mg/kg	Flu 125mg/m^2: Cy120 mg/kg: ATG 160 mg/kg	Flu 120-150 mg/m^2: Cy120 mg/kg: ATG 120 mg/kg	Flu 180 mg/m^2: Cy120 mg/kg: ATG 40 mg/kg	Flu 90 mg/m^2: Cy200 mg/kg
Type of transplant	Matched related donor	Matched related 22/ 1 antigen mismatch 4	Matched related 3: Matched unrelated 2	Matched related donor	Matched related donor
Stem cell source	BMH 97% PBSC 3%	PBSC	BMH	PBSC 90% BMH 10%	BM
aGVHD %	11.6	65	40	35	11
cGVHD %	12.9	56	80	44	25
OS (%)	83.7	77	100	75.8	79

*Flu denotes fludarabine, Cy cyclophosphamide, ATG anti-thymocyte globulin, BMH bone marrow harvest, PBSC peripheral blood stem cells, aGVHD acute graft versus host disease, cGVHD chronic graft versus host disease, OS overall survival

establishing a standard of care conditioning regimen in high-risk AA patients.

ACKNOWLEDGMENTS

The authors thank Professor Judith Marsh, Department of Haematological Medicine, King's College Hospital, London, for sharing her valuable expertise during the course of this research and her critical review that greatly improved the manuscript.

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: Q.N.C. and R.I. designed the study and contributed equally to the article. All authors contributed to data collection, analysis, and literature review and reference citation.

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