IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Allogeneic Stem Cell Transplant in Hematological Disorders: A Decade of Experience

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Received: 29, Jul, 2023
Accepted: 08, Aug, 2023

ABSTRACT

Background: Allogeneic hematopoietic cell transplantation (allo-HCT) is a complex procedure with the potential to provide curative treatment for various hematological disorders. This study aims to evaluate the outcomes of allo-HCT in hematological diseases and identify significant complications in a single-center setting.

Materials and Methods: We conducted a retrospective analysis of 180 patients with hematological diseases who underwent allo-HCT between January 2011 and December 2021. Key outcomes, including indications for transplantation, overall survival, engraftment time, relapse rates, graft-versus-host disease (GVHD), and transplant-related mortality (TRM) were assessed.

Results: The most common indications for allo-HCT were benign hematological diseases, particularly aplastic anemia, and thalassemia major. Despite the majority of patients receiving fully matched transplants, acute GVHD was observed in 30% of the cohort. Graft failure occurred in 13 patients, with primary and secondary graft failure rates of 1.6% and 5.5%, respectively. Sepsis emerged as the primary cause of non-relapsed mortality at day 100 and beyond. The overall survival rate in this study was 62%, with 79% of patients disease-free on their last visit.

Conclusion: This study provides valuable insights into the treatment strategies and patient care of allo-HCT for hematological disorders by offering a comprehensive overview of multiple relevant outcomes. The findings underscore the significance of addressing complications and risk factors associated with allogeneic transplantation, including GVHD and infections. Future research should focus on further optimizing transplantation techniques to minimize complications and enhance patient survival.

Keywords: Allogeneic stem cell transplant; Graft–vs-host disease (GvHD); Transplant-related mortality; Hematological diseases

INTRODUCTION

Over 60 years ago, hematopoietic stem cell transplantation was suggested as a possible treatment. For some patients with hematologic malignancies, benign hematologic diseases, immunodeficiencies, and genetic disorders, allogeneic hematopoietic cell transplantation (allo-HCT) is a curative treatment option (i.e., hemoglobinopathies and storage diseases)[1]. Everybody having a donor has become a reality over the past 20 years thanks to the development of haploidentical transplant techniques[2]. Significant progress has been made in recent years in lowering transplant-related mortality, modifying the graft-versus-leukemia effect to prevent or treat relapse, and establishing allo-HCT as a framework for novel cellular therapies[3,4]. Numerous factors, including donor availability, social difficulties, economic conditions, and health care systems, influence whether a patient eligible for allo-HCT receives it[5].

Beta thalassemia is more common in our nation than in the West due to consanguineous marriages. Along with aplastic anemia, it continues to be one of the main indications for stem cell transplantation. Additionally, because of consanguineous marriages and large families, human leukocyte antigen (HLA) identical sibling donors are more readily available here in Pakistan compared to the Western population[6].

The outcome of allo-HCT is influenced by several variables, including disease, patient, and donor-specific variables. Remission status, responsiveness to therapy, the use of a particular transplant type, the source of the hematopoietic stem cells, conditioning, and immunosuppressive regimens are some disease-specific considerations. Comorbidities, group-specific ages, such as pediatrics versus adults, and infectious diseases are examples of patient-specific factors. Donor-related characteristics include age, infectious diseases, sex, and stem cell source. All of these variables may have an impact on disease management, transplant-related mortality (TRM), and overall survival (OS); nonetheless, for allo-HCT, the level of HLA matching between the donor and recipient continues to be one of the most important variables for HCT outcome[7, 8].

In this study, we discuss our own allo-HCT experience from the last ten years. Our center is one of the few in Pakistan that is capable of doing hematopoietic transplants, including allo-HCT. It was founded in 2004, and since then we have performed well over 400 transplants, including pediatric and adult patients with various hematological diseases. Additionally, there have been many developments and advances in the field over the past ten years. The present study attempted to assess our performance and identify whether our expectations are met. We also explored disease complications occurring in patients and what may be done to alleviate them. We aimed to advance the field by identifying key components that improve outcomes for our center and future replication at other institutions.

MATERIALS AND METHODS

Patient population

All patients with non-malignant and malignant hematological disorders underwent allogeneic stem cell transplantation from HLA-matched donors at Aga Khan Hospital, Karachi, between Jan 2011 and Dec 2021. The study received approval from our institutional board and Ethics Committee (ERC Ref # 2023-8220-23979). All procedures carried out in the study were in accordance with the ethical principles of the 1964 Helsinki Declaration and its later amendments. Informed written consent was taken from all the participants prior to the data collection.

Pre-transplant workup

Besides HLA typing, complete blood counts, liver and kidney function tests (creatinine, electrolytes), and infectious disease profile (consisting of hepatitis B surface antigen, hepatitis C antibody, HIV antibody, Cytomegalovirus) chest Xray along with blood grouping, urine analysis coagulation testing, and psychiatric evaluation were performed in all donors. For patients, screening included all the above-mentioned investigations along with pulmonary function tests, echocardiography, and dental evaluation.

Stem cell mobilization

All donors were given granulocyte–colony-stimulating factor (G-CSF) at a dose of 5-10 µg/kg/twice daily for five days before harvest. Patients with donors less than five years old received bone marrow only as the stem cell source. In patients with aplastic anemia, peripheral blood, as well as bone marrow stem cells, were the preferred source. In all other conditions, peripheral blood progenitor cells only were used as the source of stem cells.

Conditioning regimen

Patients with Thalassemia, Acute Myeloid Leukemia, Chronic Myeloid Leukemia, Biphenotypic Leukemia, and Philadelphia negative Acute Lymphoblastic Leukemia received Busulfan (3.2 mg/kg for four days) and Cyclophosphamide (60 mg/kg/day for two days) as conditioning chemotherapy. Class III thalassemic patients received (Thalassemia Protocol) conditioning with hyperchelation protocol

which consisted of deferoxamine 40 mg/kg, hydroxyurea 30 mg/kg, and azathioprine 3 mg/kg daily between day -45 and day -11 before transplantation. From day -17 till day -13, Fludarabine was administered at a dose of 36mg/m^2/day. ATG 1.33 mg/Kg/day total was administered on D-12 to D-10. From day -9 to day -6, Busulfan was started at 1.6-2.4 mg/kg/dose (depending upon the weight of the patient) for four days (total 14 doses) followed by cyclophosphamide 50 mg/kg for four days. Total body irradiation (1.5GY x twice a day) and Cyclophosphamide (60 mg/kg/day for two days) were used in patients with Philadelphia-positive Acute Lymphoblastic Leukemia and those with one-antigen mismatch donors. In Aplastic anemia, rabbit anti-thymocyte globulin (10 mg/kg/day for three days) and Cyclophosphamide (50 mg/kg/day for four days) were used. Patients with Fanconi's anemia received conditioning with Fludarabine (30 mg/kg/day for five days), Cyclophosphamide (300 mg/m^2 for four days), and rabbit anti-thymocyte globulin (3.75 mg/kg/day for three days).

Infectious disease prophylaxis

Patients were admitted in protective isolation equipped with a HEPA filter, positive pressure, and laminar airflow ventilation. Standard prophylaxis with Ciprofloxacin (500 mg twice daily or 20-30 mg/kg/two divided doses) or levofloxacin 500mg once daily oral/ IV, Fluconazole (400mg once daily or 6 mg/kg/day) or voriconazole (200mg twice daily) and Acyclovir (400mg to 800mg oral twice daily or 250mg/m2 IV every 12 hourly) or Valaciclovir (500 mg twice daily or 10 mg/kg/twice daily) were started in all patients on day-5. All patients were provided with a neutropenic diet.

Graft versus host disease prophylaxis

Intravenous Cyclosporine was started on day -1 and doses were adjusted according to drug levels. The optimum adult range was 200-400 ng/dl. Methotrexate 15 mg/m^2 was administered on day +1, while 10 mg/m2 was given on days +3 +6, and +11. Each Methotrexate dose was given on physician approval followed by 24 hours inj. leucovorin 15mg IV, 3 doses each 6 hours apart. Post-transplant cyclophosphamide 50 mg/kg if used as Haploidentical transplant then GVHD prophylaxis to be started at Day+5. Irradiated and leukocyte-reduced blood products were used throughout admissions well as in the post-transplant period.

Assessment of engraftment

By definition, WBC engraftment is an absolute neutrophil count >500 for three consecutive days and the platelet engraftment is the platelet count >20,000 for three consecutive days without any external transfusion support.

Objectives

This study aims to evaluate the long-term outcomes of allogeneic stem cell transplantation. It will examine key parameters such as indications for transplantation, overall survival rates, survival rates for specific indications, median survival duration, engraftment time, relapse rates, graft-versus-host disease (GVHD), and transplant-related mortality. The study will also analyze infectious and noninfectious factors contributing to transplant-related mortality, including bacterial, viral, and fungal infections, graft failure, multiorgan failure, and veno-occlusive disease (VOD). By analyzing a decade of experience, the study aims to provide valuable insights for improving treatment strategies and patient care in the field of stem cell transplantation.

Statistical analysis

Continuous variables were presented as means accompanied by their corresponding standard deviations, while categorical variables were expressed as percentage frequencies. To determine the overall survival time within the overall sample, as well as for specific transplant indications, a Kaplan-Meier analysis was conducted. The calculated median survival time and interquartile range were reported for indications where the median value could be determined. However, for certain indications, the median survival time could not be calculated due to the limited number of deaths observed within those particular groups during the

observation period. Kaplan-Meier graphs were generated to visually depict the survival outcomes.
Furthermore, a multivariable logistic regression analysis was performed to assess the association between the occurrence of cytomegalovirus (CMV) infection post-transplantation and the CMV status of both the donor and recipient before transplantation. The donor and recipient's CMV status were considered as moderating factors in this regression analysis. The statistical analyses were conducted using STATA version 16 and R version 4.2.2.

RESULTS

Patient population

A total of 180 patients with hematological diseases were included in this study. The mean age of the patient cohort was 22.5 years (SD ± 13.3 years, range 1–58 years) (Table 1). Of the participants, 129 (71.7%) were male, while 51 (28.3%) were female. 75 (41.6%) patients belonged to the pediatric population, while 105 (58.3%) were adults.

HLA match and stem cell source

The human leukocyte antigen (HLA) matching status revealed that 150 patients (83.3%) had a full match (match sibling donor), 29 patients (16.1%) had a haploidentical match, and only one patient (0.5%) had a 9/10 partial match. Regarding the stem cell source, peripheral blood stem cells (PBSC) were utilized in the majority of cases (125 patients, 69.4%), followed by bone marrow (37 patients, 20.5%), and a combination of PBSC and bone marrow (18 patients, 10%) (Table 1).

CMV serostatus

Information regarding CMV serostatus was available for a subset of patients. Among the donors, 51 individuals (28.3%) were CMV-positive, five individuals (2.7%) were CMV-negative, and the CMV status was missing for 124 individuals (68.8%). Among the recipients, 151 individuals (83.8%) were CMV-positive, 12 individuals (6.66%) were CMV-negative, and the CMV status was missing for 17 individuals (9.4%) (Table 1).

Table 1: Patient demographics

Parameter	Value
Patient Population	n=180
Age, mean (SD), years	22.5 (13.3)
Gender, n (%)	
- Male	129 (71.7%)
- Female	51 (28.3%)
HLA Match, n (%)	
- Full Match	150 (83.3%)
- Haplo Match	29 (16.1%)
- 9/10	1 (0.5%)
Stem Cell Source, n (%)	
- PBSC	125 (69.4%)
- BM	37 (20.5%)
- Both	18 (10%)
CMV Serostatus	
- Donor	
• Positive	51 (28.3%)
• Negative	5 (2.7%)
• Data not available	124 (68.8%)
- Recipient	
• Positive	151 (83.8%)
• Negative	12 (6.66%)
• Data not available	17 (9.4%)

HLA: human leukocyte antigen, BM: bone marrow, PBSC: peripheral blood stem cells, CMV: cytomegalovirus

Transplant Indication and engraftment

The diverse hematological diseases represented in the patient population included aplastic anemia (45 patients, 25%), B-cell acute lymphoblastic leukemia (B-ALL) (35 patients, 19.4%), acute myeloid leukemia (AML) (33 patients, 18.3%), thalassemia major (30 patients, 16.6%), T-cell acute lymphoblastic leukemia (T-ALL) (10 patients, 5.5%), myelodysplastic syndrome (MDS) (5 patients, 2.7%), chronic myeloid leukemia (CML) (5 patients, 2.7%), mixed phenotype acute leukemia (MPAL) (3 patients, 1.6%), hemophagocytic lymphohistiocytosis (HLH) (3 patients, 1.6%), Hodgkin lymphoma (3 patients, 1.6%), chronic myelomonocytic leukemia (CMML) (2 patients, 1.11%), chronic eosinophilic leukemia (1 patient, 0.56%), Fanconi anemia (1 patient, 0.56%), myelofibrosis (1 patient, 0.56%), non-Hodgkin lymphoma (1 patient, 0.56%), pure red cell aplasia (1 patient, 0.56%), and sickle cell anemia (1 patient, 0.56%) (Table 2). The mean time for neutrophil engraftment was 14.3 days (SD ± 3.6), while the meantime for platelet engraftment was 17.2 days (SD ± 3.85).

Table 2: Transplant indications and engraftment

Transplant Indication, n (%)	
- Aplastic Anemia	45 (25%)
- B-ALL	35 (19.4%)
- AML	33 (18.3%)
- Thalassemia Major	30 (16.6%)
- T-ALL	10 (5.5%)
- MDS	5 (2.7%)
- CML	5 (2.7%)
- MPAL	3 (1.6%)
- HLH	3 (1.6%)
- Hodgkin Lymphoma	3 (1.6%)
- CMML	2 (1.11%)
- Chronic Eosinophilic Leukemia	1 (0.56%)
- Fanconi Anemia	1 (0.56%)
- Myelofibrosis	1 (0.56%)
- Non-Hodgkin Lymphoma	1 (0.56%)
- Pure Red Cell Aplasia	1 (0.56%)
- Sickle Cell Anemia	1 (0.56%)
Engraftment, mean (SD), days	
- Neutrophil	14.3 (3.6)
- Platelets	17.2 (3.8)

B-ALL: B-cell acute lymphoblastic leukemia, AML: acute myeloid leukemia, T-ALL: T-cell acute lymphoblastic leukemia, MDS: myelodysplastic syndrome, CML: chronic myeloid leukemia, MPAL: mixed phenotype acute leukemia, HLH: hemophagocytic lymphohistiocytosis, CMML: chronic myelomonocytic leukemia

Infective complications

Febrile neutropenia, a common infective complication, occurred in 113 patients (62.8%). The most prevalent organisms associated with febrile neutropenia were E. coli (22 patients, 19.4%), Pseudomonas species (13 patients, 11.5%), Klebsiella pneumonia (4 patients, 3.5%), Methicillin-resistant Staphylococcus aureus (MRSA) (7 patients, 6.19%), Staphylococcus not aureus (18 patients, 15.9%), and Stenotrophomonas maltophilia (7 patients, 6.19%) Table 3. Post-transplant infections after successful engraftment included cytomegalovirus (CMV) (48 patients, 26.6%), bacterial infections (49 patients, 27.2%), and fungal infections (12 patients, 6.6%).

Table 3: Infective complications

Febrile Neutropenia, n (%)	**113 (62.8%)**
Common Organisms of Febrile Neutropenia	
- E. coli	22 (19.4 %)
- Pseudomonas species	13 (11.5%)
- Klebsiella pneumoniae	4 (3.5%)
- MRSA	7 (6.19%)
- Staph not Aureus	18 (15.9%)
- Stenotrophomonas maltophilia	7 (6.19%)
Post-Transplant infections, n (%)	
- CMV	48 (26.6%)
- Bacterial	49 (27.2%)
- Fungal	12 (6.6%)

E.coli: escherichia coli, MRSA: methicillin-resistant staphylococcus aureus, CMV: Cytomegalovirus

Graft failure

Altogether, there were 13 cases of graft failure (7.2%). Primary graft failure occurred in 3 patients with an overall incidence of 1.6%, and secondary graft failure occurred in 10 patients with an overall incidence of 5.5%. Graft failure was more common in thalassemia major (6 patients, 46.1%), aplastic anemia (5 patients, 38.4%), MDS (1 patient, 7.6%), and B-ALL (1 patient, 7.6%) (Table 4).

Table 4: Non-Infective complication

Graft Failure, n (%)	**13 (7.2%)**
- Primary	3 (1.6%)
- Secondary	10 (5.5%)
Graft Failure by disease	
- Thalassemia Major	6 (46.1%)
- Aplastic Anemia	5 (38.4%)
- MDS	1 (7.6%)
- B-ALL	1(7.6%)
Graft vs Host Disease, n (%)	
- Acute	54 (30%)
- Chronic	27 (15%)
Grade of acute GVHD, n (%)	
- 1	11 (20.3%)
- 2	21 (38.8%)
- 3	14 (25.9%)
- 4	6 (11.1%)
- Not available	2 (3.7%)
Acute GVHD by organ involvement, n (%)	
- Gut	29 (53.7%)
- Skin	26 (48.1%)
- Liver	16 (29.6%)
Acute progressing to chronic, n (%)	25 (46.3%)
Other non-infective complications, n (%)	
- Mucositis	69 (38.3%)
- Hemorrhagic cystitis	11 (6.11%)
- VOD/SOS	10 (5.5%)
- Thrombotic microangiopathy	6 (3.33%)
- Renal Failure	4 (2.22%)
- Seizures	4 (2.22%)
- Hypertension	4 (2.22%)

MDS: myelodysplastic syndrome, B-ALL: B-cell acute lymphoblastic leukemia, GVHD: graft versus host disease, VOD/SOS: veno-occlusive disease or sinusoidal obstruction syndrome

Graft-versus-host disease (GVHD)

GVHD occurred in 54 patients (30%), with acute GVHD being more prevalent than chronic GVHD (27 patients, 15%). The grade distribution of acute GVHD was as follows: grade 1 in 11 patients (20.3%), grade 2 in 21 patients (38.8%), grade 3 in 14 patients (25.9%), grade 4 in 6 patients (11.1%), and not available in 2 patients (3.7%). Acute GVHD by organ involvement included the gut (29 patients, 53.7%), skin (26 patients, 48.1%), and liver (16 patients, 29.6%). Of those with acute GVHD, 25 (46.3%) progressed to chronic GVHD (Table 4).

Post-transplant non-infective complications

Non-infective complications included mucositis (69 patients, 38.3%), hemorrhagic cystitis (11 patients, 6.11%), veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) (10 patients, 5.5%), thrombotic microangiopathy (6 patients, 3.33%), renal failure (4 patients, 2.22%), seizures (4 patients, 2.22%), and ciclosporin-induced hypertension (4 patients, 2.22%) (Table 4).

Transplant-related mortality at D+100

Among the cohort of patients (n = 38), transplant-related mortality at day 100 (D+100) was evaluated, revealing that sepsis was the most prevalent cause, accounting for 86.5% (n = 33) of cases. Hepatic failure followed as the second leading cause, with a prevalence of 57.8% (n = 22). Other significant factors contributing to mortality included acute respiratory distress syndrome (39.5%, n = 15), graft-versus-host disease (21.0%, n = 8), renal failure (21.0%, n = 8), and relapse-related mortality (13.1%, n = 5) (Table 5).

Table 5: Survival and relapse

Transplant Related Mortality D+100, n (%)	n=38
- Sepsis	33 (86.5 %)
- Hepatic Failure	22 (57.8%)
- ARDS	15 (39.5 %)
- GVHD	8 (21.0%)
- Renal Failure	8 (21.0%)
- Relapse	5 (13.1%)
Survival	
- Alive	113 (62.78%)
- Dead	63 (35.0%)
- Lost to follow up	4 (2.22%)
Survival percentage by indication	
- AML	57.58%
- Aplastic Anemia	64.44%
- B-ALL	68.57%
- T-ALL	10%
- Thalassemia	90%
Median Survival Time, days (IQR)	
- Overall	N/A
- B-ALL	1061 (434 – N/A)
- CMML	266 (266 – N/A)
- HLH	56 (20 – N/A)
- Hodgkin Lymphoma	97 (97 – N/A)
- MDS	1063 (265 – 1063)
- MPAL	72 (72 – N/A)
- T-ALL	139 (47 – 436)
Relapse of the Primary Disease	n=176
- Yes	37 (21.02%)
- No	139 (78.9%)

ARDS: acute respiratory distress syndrome, GVHD: graft versus host disease, B-ALL: B-cell acute lymphoblastic leukemia, AML: acute myeloid leukemia, T-ALL: T-cell acute lymphoblastic leukemia, MDS: myelodysplastic syndrome, MPAL: mixed phenotype acute leukemia, HLH: hemophagocytic lymphohistiocytosis, CMML: chronic myelomonocytic leukemia

Survival

In our analysis, we followed up with 176 out of 180 patients beyond the 100-day mortality mark. Among these patients, 113 (62.78%) were alive at the time of follow-up (Figure 1), while 63 (35.0%) had unfortunately passed away, and four (2.22%) were lost to follow-up. The survival percentage varied by disease indication, with AML at 57.58%, aplastic anemia at 64.44%, B-ALL at 68.57%, T-ALL at 10%, and thalassemia at 90% (Figure 2). The causes of mortality in these patients were consistent with those observed within the 100 days, with sepsis being the most common cause and accounting for a significant proportion of deaths.

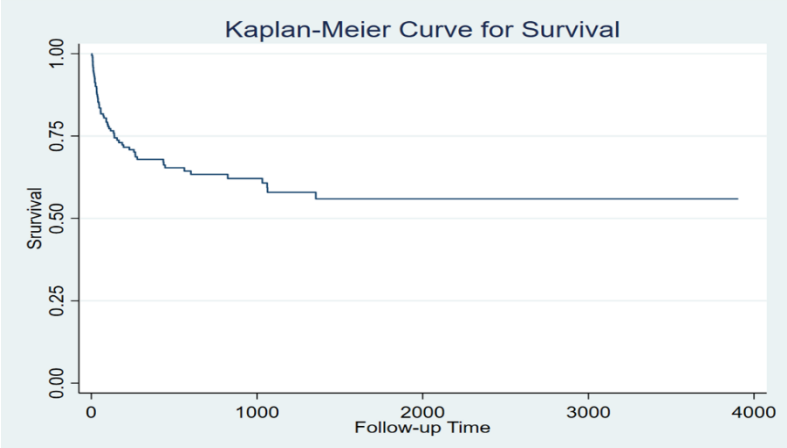

Figure 1. Overall survival (n=176)

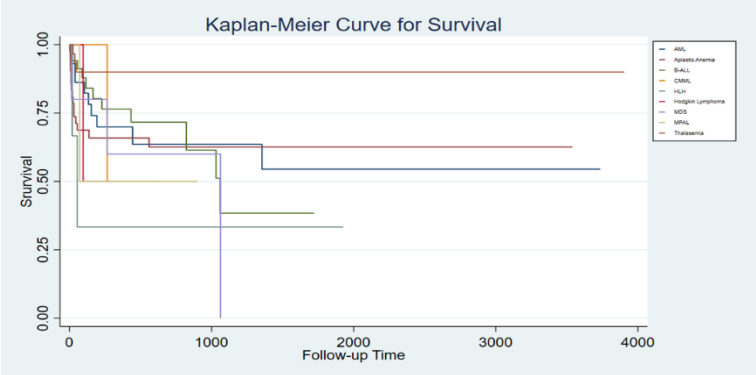

Figure 2. Overall survival by indications

Median survival time

Due to the small number of fatalities that took place within the sample, it was not possible to determine the median survival time for the entire sample. Due to the same limitation, the median survival could only be calculated for a small subset of the indications. For specific diseases, the median survival time was 1061 days (interquartile range [IQR]: 434 – not available) for B-ALL, 266 days (IQR: 266 – not available) for CMML, 56 days (IQR: 20 – not available) for HLH, 97 days (IQR: 97 – not available) for Hodgkin lymphoma, 1063 days (IQR: 265–1063) for MDS, 72 days (IQR: 72 – not available) for MPAL, and 139 days (IQR: 47–436) for T-ALL (**Table 5)**.

Relapse of the primary disease

Out of the 176 patients who had follow-up data, 37 patients (21.02%) experienced a relapse of the primary disease, while 139 patients (78.9%) (Table 5).

DISCUSSION

Allogeneic stem cell transplantation is a complex procedure known for its potential complications; however, it holds promise as a curative treatment for various hematological diseases. The associated morbidity and mortality, which have significantly decreased over the past three decades, must be taken into consideration while weighing the advantages and disadvantages of hematopoietic stem cell transplantation. In this study, we aim to assess the outcomes of ASCT in hematological diseases and identify associated complications.

A retrospective analysis was conducted on a cohort of 180 patients with diverse hematological disorders treated between January 2011 and December 2021. Contrary to international studies, where malignant diseases such as acute leukemias are the primary indication for transplantation[9], our findings demonstrate that allo-HCT is predominantly used for benign diseases like aplastic anemia and beta-thalassemia major. Nevertheless, a noticeable shift has occurred in recent years, as B-ALL has surpassed beta-thalassemia as the second most common indication, as seen in a previous study conducted at our institution[6].

In contrast to global trends, the majority of our patients received allo-HCT from matched sibling donors due to the absence of a centralized donor registry in our country. Peripheral blood emerged as the primary source of stem cells, in line with worldwide practices, owing to its ease of donation, improved engraftment, and graft-versus-leukemia (GVL) effect[10]. However, in select cases where graft-versus-host disease (GVHD) needed to be minimized, bone marrow was utilized either alone or in combination with peripheral blood stem cells (PBSC). Notably, our institution has only recently introduced cryopreservation for the unmanipulated graft source in 2023.

Infective complications

Bacterial

Infective complications pose a significant concern in allo-HCT, often leading to morbidity and mortality, even in long-term survivors. While microbiologically defined infections (MDIs) account for only 30% to 40% of cases of neutropenic fever, certain pathogens have been consistently reported[11]. Gram-negative bacilli (GNB), such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, are frequently encountered, while Gram-positive cocci (GPC), including Enterococcus, Streptococcus, and Staphylococcus species, are common pathogens. Infections have been identified as a major cause of death, representing 20.7% of all deaths in a retrospective analysis[12], with infection being the second most frequent cause of death among patients alive one year after hematopoietic cell transplantation (HCT). The risk of opportunistic infection during the pre-engraftment period varies based on factors such as anticancer drug regimens, conditioning intensity, and acute graft-versus-host disease (GVHD). Bacterial infections primarily originate from the normal gastrointestinal flora and indwelling vascular catheters, with GNB commonly found in the former and GPC in the latter[13].

Our study revealed that 62.8% of patients experienced febrile neutropenia during the pre-engraftment period (days 0 to days 15–45), with Escherichia coli and Pseudomonas species being the most frequently identified causative organisms. These findings can be attributed to the fact the myeloablative conditioning regimen was the predominant one in our center. These findings also align with similar studies that reported Escherichia coli as the most commonly isolated pathogen in blood cultures of febrile neutropenic patients[14]. In some cohorts, Pseudomonas spp. and Acinetobacter spp. were responsible for a significant proportion (43%) of sepsis episodes[15]. Notably, despite prophylactic antibiotic use, the risk of infections remains high, which can be attributed to the immunosuppressive effects of conditioning regimens. Furthermore, the risk of infections is heightened in the presence of active GVHD due to incomplete immune reconstitution and ongoing immunosuppressive therapy.

Virus

Cytomegalovirus (CMV) infection is a significant concern following allo-HCT due to its potential for fatality[16]. In this research study, CMV infection was responsible for 2.2% of non-relapsed mortality cases, and it can occur through reactivation or reinfection

post-transplantation. Among the patients analyzed, 26.67% experienced CMV infection after transplantation. However, a multivariable logistic regression analysis considering the CMV status of both the donor and recipient before transplantation revealed that neither of these factors had an impact on the likelihood of developing an active CMV infection (p-values 0.764 for both). CMV infection ranges from asymptomatic antigenemia or DNAemia to severe diseases affecting various organs. CMV reactivation can also indirectly lead to complications such as graft failure and immunosuppression, resulting in concurrent bacterial and fungal infections. In this study, 28 patients developed bacterial infections alongside CMV infection, and 4 cases were associated with graft failure. Notably, the recent approval of letermovir, unavailable in this study, has significantly reduced the incidence of CMV infection and disease following allo-HCT [17].

Within the first year following allo-HCT, a substantial proportion of patients (ranging from 20% to 53%)[18] commonly experience reactivation of varicella zoster virus (VZV). Remarkably, although the vast majority of our patients were already seropositive for VZV prior to transplantation, VZV reactivation was observed in only two cases. This lower incidence could potentially be attributed to the rigorous adherence to post-transplant antiviral prophylaxis, supported by compelling evidence from multiple randomized studies that demonstrated its efficacy in reducing VZV reactivation to ≤5% during the initial year after HCT[18,19]. However, it is important to acknowledge that, unlike our allo-HCT patients, our autologous transplant patients did experience a few localized outbreaks, but their condition did not progress to disseminated infection.

BK virus infection emerged as a notable viral factor within our patient cohort, specifically in the context of hemorrhagic cystitis. Among the eleven cases of hemorrhagic cystitis observed, four were found to be associated with BK virus infection. In contrast, the incidence of other common viral respiratory infections was sporadic in our post-transplant patient population. In response to the recent COVID-19 pandemic, we implemented a pre-transplant PCR testing strategy, deferring transplantation for individuals testing positive. Following transplantation, we advised patients to postpone vaccination until discontinuation of immunosuppressive medication. In the interim, we recommended the use of masks and a reduction in social contact. During this observation period, three patient deaths attributable to COVID-19 were recorded within our cohort, while several individuals exhibited mild COVID-19 symptoms without comprehensive documentation.

Fungi

Aspergillus species are recognized as the predominant causative agents of invasive fungal infections (IFIs) in patients with hematologic diseases, closely followed by Candida spp. and less commonly encountered fungi. These patterns of fungal pathogens remain consistent in the context of hematopoietic stem cell transplantation (HSCT)[20]. In our study, the occurrence of fungal infections was not uncommon, with a total of 12 cases reported, corresponding to 6.6% of the transplanted patients. Among these cases, three were diagnosed with aspergillosis, three with candidiasis, while the remaining cases involved rare fungal species. The incidence rates align with those reported in studies conducted within the same region[21]. Notably, this can be attributed to the availability of advanced diagnostic modalities, such as β-D-glucan and Galactomannan testing, at our institution, enabling early detection and prompt escalation of treatment. Furthermore, our standard prophylactic regimen incorporating voriconazole proved effective in preventing fungal infections among most of our patient population.

GVHD

Graft-versus-host disease (GVHD) is a prevalent complication following allo-HCT and has substantial implications for patient-reported quality of life (QOL), physical functioning, and clinical outcomes[22]. The classical form of acute GVHD typically manifests within the initial 100 days post-transplantation and primarily affects the skin, liver, and gastrointestinal tract.

Recent progress in the field can be partially attributed to improved donor-recipient matching, leading to a reduced incidence of GVHD, as well as

advancements in GVHD treatments and supportive care.

In our study, despite the majority of patients undergoing fully matched transplants, we observed that 30% (54 patients) developed GVHD, with the majority of cases being Grade 2 (38.8%), with 46% of those cases progressing to chronic GVHD Table 4. Notably, patients who received bone marrow (BM) as the graft source exhibited a lower incidence of GVHD, with only 4 out of 54 cases developing the condition. Similarly, those who underwent ATG-based conditioning regimens also experienced a lower risk. Conversely, the use of peripheral blood stem cells (PBSC) as a graft source significantly increases the risk of GVHD[23]. In our study, 50 out of 54 patients who developed GVHD had PBSC as the source of stem cells.

Despite the progress made, corticosteroids continue to be the primary choice for initial treatment, and unsatisfactory outcomes have been observed in cases of steroid-refractory GVHD. Notably, the use of ruxolitinib has shown promise in recent years[24,25], and we successfully employed this treatment in our patients with steroid-refractory GVHD, resulting in the discontinuation of long-term steroid use.

Furthermore, the utilization of post-transplant cyclophosphamide (PTCy) or αβTCR depletion to eliminate alloreactive T cells has facilitated the utilization of haploidentical donors in older recipients with hematologic malignancies, thereby mitigating the risks of GVHD and graft rejection[26]. However, it is important to note that our institution does not currently employ PTCy or T-cell depletion as standard practice.

Graft failure

Primary graft failure (PGF) and secondary graft failure (GF) occur in a significant proportion of patients undergoing allo-HCT), with a reported incidence of primary GF is 0.6–9.6% and secondary GF is 1.7–5.0% [27].

In our study, graft failure was observed in 13 patients, with three cases (1.6%) attributed to primary graft failure and 10 cases (5.5%) classified as secondary graft failure among all transplant patients. Thalassemia was detected in six patients, followed by aplastic anemia in five cases.

The primary cause of graft failure is the mismatch between the human leukocyte antigen (HLA) profiles of the donor and recipient. The incidence of primary graft failure is significantly lower in patients who receive peripheral blood stem cell (PBSC) grafts compared to those who receive bone marrow grafts[10]. This observation can be attributed to our practice of utilizing HLA-matched sibling donors as the primary source of donor cells. In cases of haploidentical transplantation, we ensure thorough screening for anti-HLA antibodies before proceeding with the transplant. Furthermore, PBSC grafts were predominantly used (70%) in our transplanted patients, which may have contributed to the reduced incidence of graft failure.

Other complications

In addition to graft-versus-host disease (GVHD) and graft failure, our research has identified several non-infective complications associated with allo-HCT, summarized in Table 4. Among these complications, mucositis emerged as the most common. In our study, mucositis was predominantly linked to Cy-TBI (37.68%) and Bu-CY (28.99%) conditioning regimens. Notably, the prevalence of mucositis was 52.00% for Cy-TBI and 47.62% for Bu-CY.

The second most prevalent conditioning-related toxicity was hemorrhagic cystitis, with an incidence of 11%. Although the reported incidence of this complication ranges from 12% to 36%[28], it is more pronounced in myeloablative conditioning regimens containing cyclophosphamide[29], which aligns with our findings. Our center follows the standard of care by implementing mesna for the prevention of this complication.

Within our study cohort, we observed VOD in up to 10 patients, corresponding to an incidence rate of 5%, while international studies show significant variability of 5% to 60% and historically high mortality rates of up to 80% [30]. Unfortunately, due to the limited availability of defibrotide in our country, we were only able to secure the medication for a single patient. Regrettably, despite receiving defibrotide, this patient succumbed to the disease. Additionally, among the remaining four patients who did not receive defibrotide, all experienced mortality. It is noteworthy that these four patients

passed away before reaching day 100, while the patient receiving defibrotide survived for a longer duration (445 days) compared to the others.

Transplant-associated thrombotic microangiopathy (TA-TMA) was observed in six patients, with only two surviving through supportive interventions, such as plasma exchange and transitioning to non-calcineurin-based immunosuppressive agents like mycophenolate mofetil (MMF).

Furthermore, our study cohort exhibited several late complications. These included bilateral cataracts, cardiomyopathy, ciclosporin-induced neurotoxicity presenting as bilateral lower limb weakness, autoimmune hemolytic anemia, squamous cell carcinoma as a secondary malignancy post-transplant, pulmonary hemorrhages, and pulmonary fibrosis, with one case of each complication observed.

Survival and relapse

The overall survival rate in our cohort was 62%, as depicted in Figure 1. Among the 180 patients included, 37 (21%) experienced a relapse of the primary disease. A retrospective analysis conducted by the European Society for Blood and Marrow Transplantation (EBMT) investigated overall survival rates between 2010 and 2016, revealing rates ranging from 62% to 83% [12]. These findings are consistent with our results and surpass the rates reported by Kong et al. in 2021[31], which were approximately 52%. A comprehensive summary of the survival rates in terms of percentages and median survival time is presented in Table 5. Notably, benign hematological diseases, such as thalassemia (90%) and aplastic anemia (64%), demonstrated higher survival rates compared to malignant diseases, including AML (57%). Surprisingly, B-ALL exhibited a higher survival rate (68%) than other malignant diseases. These findings corroborate our previous study[6], reinforcing the consistency of observations.

The cumulative incidence risk of transplant-related mortality at day +100 in our cohort was 21.1%, with relapse-related mortality accounting for 2.7%. Although the incidence was higher in our cohort, sepsis remained the primary cause of death even beyond day 100. These results differ from other studies[12,31], where relapse-related mortality constituted 50% of cases, followed by graft-versus-host disease (GVHD) at 8-20%. This contrast may be attributed to a broader prevalence of antibiotic resistance resulting from the easy availability of over-the-counter medications and the utilization of fully HLA-matched donors in our cohort, leading to reduced incidences of GVHD and relapse-related deaths.

Strength and limitations

This research has several strengths. It examines a large sample size over a decade of allogeneic stem cell transplantation outcomes and complications in different hematological disorders, offering a holistic overview. Its broad patient sample increases its external validity, and long-term follow-up provides insight into the procedure's durability. Additionally, it studies the impact of graft sources and conditioning regimens on graft-versus-host disease (GVHD) and recognizes non-infective complications (e.g. mucositis, hemorrhagic cystitis). Overall, this research offers a comprehensive understanding of transplantation outcomes and practical implications for clinicians and researchers.

This study has strengths yet also certain limitations. It is single-centered, introducing bias and limiting generalizability. The large sample size contributes but results should still be considered in light of the center's practices and patient population. Data is retrospective, which may introduce bias and incomplete/missing data. In addition, the study acknowledges a lack of defibrotide for VOD and letermovir for CMV infection, while advanced treatments, such as PtCy and T-cell depletion, were not available. These may have improved outcomes.

CONCLUSION

Allogeneic stem cell transplantation is a complicated procedure associated with several complications and potential hope for a cure. In the past decade, greatly improved conditioning regimens and GVHD minimization strategies have been developed and introduced; however; there is still considerable room for improvement. This study summarizes our accomplishments and the various complications that can occur during allogeneic

transplantation, as well as compares our experience to international trends. It emphasizes the importance of frequent monitoring and management of complications. Our center strives to keep up with recent advances, but there are still many gaps and challenges to be addressed. Advanced technologies such as bispecific antibodies, CAR-T cell therapy, and gene editing have altered the treatment landscape; however, allogeneic transplantation remains an important option, especially in resource-constrained countries.

ACKNOWLEDGEMENTS

We are grateful to Aga Khan University Hospital's Department of Oncology, specifically the Bone Marrow Transplant Unit, for their invaluable support of our project. In particular, we would like to thank nurses Noureen Alani and Bakhtawer Munir for their extraordinary contributions beyond their roles; they were a blessing to the BMT Unit's patients.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in public, commercial, or not-for-profit sectors.

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