RESEARCH ARTICLE

Association of early donor chimerism status with survival outcomes in post allogeneic haematopoietic stem cell transplant patients of nonmalignant diseases

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Abstract

Objective: To highlight the association of early donor chimerism status at 2nd month with various survival outcomes.

Method: The retrospective study was conducted at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan, and comprised patient data from January 2011 to July 2016. Data related to participants who underwent human leukocyte antigen-matched transplants for bone marrow failure syndrome and beta thalassemia major. Short tandem repeat-based polymerase chain reaction was used to assess donor chimerism status. Overall survival, disease-free survival, relapse-free survival, and graft versus host disease-free survival rates were noted. Data was analysed using SPSS 23.

Results: Of the 106, 64(60.4%) had bone marrow failure syndrome and 42(39.6%) had beta thalassemia major. The overall median follow-up was 13.53 months (range: 1.81-62.73 months). Early donor chimerism status was associated with overall survival (p=0.02) and disease-free survival (p=0.01). Mixed donor chimerism was less hazardous in terms of overall survival (p=0.04) and disease-free survival (p=0.02).

Conclusion: Early mixed donor chimerism contributed to optimal survival in nonmalignant disease.

Keywords: Hematopoietic stem cell transplantation, Nonmalignant diseases, Survival outcome, Conditioning regimen.

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Introduction

Haematopoietic stem cell transplantation (HSCT) is a curative treatment for certain nonmalignant haematological diseases, like haemoglobinopathies, bone marrow failure syndromes (BMFS), immune deficiencies and metabolic disorders.[1] Patient monitoring is vital for the management of post-transplant complications, such as disease relapse, graft failure and graft versus host disease (GVHD). In routine, monitoring of these patients includes blood counts, blood morphology, bone marrow and donor chimerism analysis.

In nonmalignant diseases, the donor chimerism status is assessed to know the engraftment status. Chimerism is the coexistence of the donor and recipient haematopoietic cells in the patient's blood and bone marrow.[1] Chimerism status can be evaluated in whole blood/bone marrow and cell subsets of lymphoid and myeloid lineages.[1,2] Each of these has its importance with underline disease and sensitivity of chimerism assessment.

Donor chimerism is assessed at regular intervals such as at 30, 60, 100, 180, 270 and 365 days.[3] After one year, it is generally assessed every year for up to five years. In addition to this time-point, chimerism analysis can be advised to assess graft status as per need. It is desirable to have complete donor chimerism in post-allo-HSCT patients. In the case of nonmalignant haematological diseases, stable mixed donor chimerism is acceptable if it rectifies disease-specific measures, such as transfusion dependence in beta-thalassemia major (BTM) and bone marrow failure syndrome.[4-6]

Chimerism status can help in precluding imminent graft failure and improving disease status by appropriate and timely interventions, such as donor lymphocyte infusion (DLI) and immunomodulation.[7-11] Complete donor chimerism is also considered to be associated with GVHD, which is one of the major complications in post-allo-HSCT patients.[1] Immunosuppressive drugs are the mainstay to prevent GVHD in the post-transplant phase. In nonmalignant diseases, it continues up to 6-9 months after which tapering begins. Immunosuppression is stopped after sustained complete donor chimerism (CDC) or stable mixed chimerism (SMC) with disease-free status and absence of GVHD.

Conditioning regimen and GVHD prophylaxis are meant for immune suppression and to prevent GVHD. The conditioning regimen used in nonmalignant diseases is myeloablative, reduced intensity or nonmyeloablative. In the case of myeloablative conditioning, it is expected that patient will achieve CDC status[1] Reduced-intensity or nonmyeloablative conditioning regimen increase the incidence of mixed donor chimerism (MDC) in post-allo-

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HSCT with an increased risk of graft failure and relapse.

The current study was planned to determine the association of donor chimerism status with survival outcomes in post-allo-HSCT participants of nonmalignant diseases, and to assess the impact of conditioning regimen and GVHD prophylaxis on donor chimerism status in participants with nonmalignant diseases.

Materials and Methods

The retrospective study was conducted at the Armed Forces Bone Marrow Transplant Centre (AFBMTC), Rawalpindi, Pakistan, and comprised data from January 2011 to July 2016 related to patients who underwent HLA-matched related transplants for BMFS and BTM at the AFBMTC. After approval from the institutional ethics review board, the sample size was estimation on the basis of a 1992 study.[12] Data was included only of BMFS and BTM subjects who underwent allo-HSCT and had been fully HLA-matched with their donors. Those excluded were patients of other nonmalignant and malignant diseases, those with primary graft failure, and patients who were not evaluated for chimerism status at 1 year. The donor chimerism status at second month was used for evaluation and the cut-off date for follow-up was July 30, 2016.

Conditioning and GVHD prophylaxis were grouped to investigate their association with the chimerism status in the second post-transplant month. Conditioning regimens were categorised into five groups: with cyclophosphamide (Cy) vs without Cy; with fludarabine (Flu) vs without Flu; with anti-thymocyte globulin (ATG) vs without ATG; with busulphan vs without busulphan; and with thiotepa vs without thiotepa. GVHD prophylaxis was grouped as: cyclosporine (CSA) alone (yes vs no; with methotrexate (MTX) vs without MTX; and with steroids vs without steroids.

Chimerism analysis was performed with gold standard Short tandem repeat-based polymerase chain reaction (STR-based PCR).[13] In terms of chimerism status, the presence of only donor STR profiles in whole blood was characterised as CDC. When donor and recipient STR profiles coexisted in the whole blood, it was considered MDC. Mixed donor chimerism was further divided into two categories: increased MDC in which the recipient's STR profile was more prominent than the donor's; and decreased MDC in which the donor's STR profile was more prominent than the recipient's.

Recurrence of disease-specific measures, such as platelets and red cell concentrate (RCC) transfusion dependence in BMFS and BTM, respectively, was defined as relapse.

Acute and chronic GVHD data was obtained from the clinical records. Acute GVHD (AGVHD) was diagnosed by the presence of skin rash, loose stools and jaundice in early post-transplant phase (≤100 days), confirmed with or without biopsy, and graded according to the Glucksberg criteria.[14] Chronic GVHD (CGVHD), clinically diagnosed as per the National Institutes of Health (NIH) criteria, was divided into limited and extensive diseases.[15]

Participants with increased MDC along with transfusion dependence were recommended for the second transplant. Participants with decreased MDC without transfusion dependence remained untreated.

Data was analysed using SPSS 23. Survival outcomes, such as overall survival (OS), disease-free survival (DFS), relapse-free survival (RFS) and GVHD-free survival were calculated using Kaplan-Meier. Survival outcomes were compared with chimerism status at 2 months, adjusted for the underline diagnosis, by using the Kaplan-Meier Log Rank test. To avoid competing for risk effect in determining RFS, those participants who died before relapse were excluded from the analysis.[16] Cox proportional hazard was used to estimate MDC risk with associated survival outcomes. Fisher's exact or chi-square tests were used, with two-sided *p*-values, for comparison of conditioning reagent and GVHD prophylaxis with chimerism status at 2 months post-allo-HSCT. Binary logistic regression was used to find the odds ratio (OR) of mixed chimerism due to conditioning reagent and GVHD prophylaxis. Binary logistic regression was performed in variables with p=0.1.

Results

Of the 106, 64(60.4%) had BMFS and 42(39.6%) had BTM. The overall median follow-up was 13.53 months (range: 1.81-62.73 months); 41.7863 months (range: 1.81-48.62 months) in participants with MDC, and 33 months (range: 1.81-62.73 months) in participants with CDC. The median age of the recipients was 14.9 years (range: 1-50 years) and that of the donor was 15 years (range: 1-50 years). Haematopoietic stem cell source was bone marrow in 67(63.2%) participants, peripheral blood stem cell in 10(9.4%), and bone marrow plus peripheral blood stem cell in 29(27.3%) participants. Stem cell dose was infused at a median dose of $5x10^8$ cells (range:5-10.8 $x10^8$ cells). Median duration for neutrophil and platelet recovery was 13 days (range: 10-30 dys) and 22 days (range: 14-80), respectively. The median follow-up of the participants with MDC was 45.9 months (range: 2-48.6 months) and the median follow-up of the participants with CDC was 36.1 months (1.8-62.7). Of the total, donor chimerism status at second month post-allo-HSCT was available for 94(88.7%) participants. Pre-transplant and post-transplant characteristics with donor chimerism status at 2 months in nonmalignant

diseases were noted in detail (Tables 1-2).

The first group of conditioning regimens, with-ATG vs without-ATG, was independent of chimerism status (p=0.06). Participants who achieved CDC with ATG were 51(60.7%) and without-ATG were 33(39.3%). MDC was observed in 3(30%) of the with-ATG group and those who in the without-ATG group was 7(70%). In the second group, with-Flu vs without-Flu, the association was not significant (p=0.16). In the with-Flu subgroup, CDC was found in 70(83.3%) participants and MDC in 10(100%). In the without-Flusubgroup, CDC was detected in 14(16.7%) participants, while none of them were with MDC status. A significant association was found in the third group, with-busulphan vs without-busulphan (p=0.05). In the with-busulphan subgroup, CDC was found in 32(38.1%) participants and MDC in 7(70%). In the without-busulphan subgroup, CDC was found in 52(61.9%) and MDC in 3(30%) participants. No significant association was observed in the fourth group, with-thiotepa vs without-thiotepa (p=0.18). In the with-thiotepa subgroup, CDC was found in 25(29.8%) participants and MDC in 1(10%). In the without-thiotepa subgroup, CDC was found in 59(70.2%) and MDC in 9(90%) participants. The fifth group, with-FluCyATG vs without-FluCyATG, was also independent of chimerism status (p=0.10). CDC status in with-FluCyATG was 48(57.1%) and MDC in 3(30%) participants. In the without-FluCyATG subgroup, CDC was in 36(42.9%) participants and MDC in 7(70%). The binary logistic regression model was not significant (p=0.56) to assess OR for MDC in the first and third groups.

Table-1: Pre-transplant characteristics with donor chimerism status at 2 month in nonmalignant disease.

Characteristics (n =94)	**BMFS** n (%)		**BTM** n (%)	
	CDC (n=52)	**MDC** (n=3)	**CDC** (n=32)	**MDC** (n=7)
Patient Gender				
Male	44(84.6)	3(100)	18 (56.3)	6 (85.7)
Female	8(15.4)	0 (0)	14 (43.8)	1 (14.3)
Donor Gender				
Male	37 (71.2)	1 (33.3)	14 (43.8)	3 (42.9)
Female	15 (28.8)	2 (66.7)	18 (56.3)	4 (57.1)
Donor-Recipient Gender Mismatch				
Gender-Matched	31 (59.6)	01 (33.3)	19 (59.5)	2 (28.6)
Male Recipient / Female Donor	14 (26.9)	02 (66.7)	8 (25)	4 (57.1)
Female Recipient / Male Donor	7 (13.5)	0 (0)	5 (15.6)	1 (14.3)
Patient Age (Mean)	21.8 ±8.6	25.3±13.6	4.9±3.9	4.2±1.8
Donor Age (Mean)	23.6±11.3	26±17.3	6.4±5.7	8.5±4.07
Blood Group ABO Mismatch				
Complete ABO Match	37 (71.2)	2 (66.7)	20 (62.5)	6 (85.7)
Major Mismatch	8 (15.4)	0 (0)	9 (28.1)	1 (14.3)
Minor Mismatch	7 (13.5)	0 (0)	2 (6.3)	0 (0)
Bidirectional Mismatch	0 (0)	1 (33.3)	1 (3.1)	0 (0)

CDC: complete donor chimerism, MDC: Mixed donor chimerism,
BMFS: Bone marrow failure syndrome, BTM: Beta thalassemia major.

Table-2: Transplant and post-transplant characteristics with donor chimerism status at 2 months in nonmalignant disease (n=94).

Characteristics	**BMFS** n (%)		**BTM** n (%)	
	CDC (n=52)	**MDC** (n=3)	**CDC** (n=32)	**MDC** (n=7)
Stem Cell Source				
BM	31 (59.6)	1 (33.3)	24 (75)	5 (71.4)
PBSC	2 (3.8)	0 (0)	6 (18.8)	1 (14.3)
BM plus PBSC	19 (36.5)	2 (66.7)	2 (6.3)	1 (14.3)
Stem cell Dose	4.9±1.25	4.44±0.1	5±0.8	4.8±0.7
Neutrophil Engraftment (Mean)	14.2±3.5	12.33±0.5	14.1±1.9	16.2±6.2
Platelet Engraftment (Mean)	22.6±8.9	17±1	28.1±12.1	29.8±16.3

BM: Bone marrow, PBSC: Peripheral blood stem cells, CDC: complete donor chimerism, MDC: Mixed donor chimerism, BMFS: Bone marrow failure syndrome, BTM: Beta thalassemia major.

Association of donor chimerism status and the first group of GVHD prophylaxis, CSA-alone vs CSA-with-MTX or steroids was not significant (p=0.35). In the CSA-alone subgroup, participants who achieve CDC and MDC were 38(45.2%) and 3(30%), respectively. In CSA-with-MTX or steroids subgroup, CDC was in 46(54.8%) and MDC in 7(70%) participants. The second group, with-MTX vs without-MTX, was also independent of donor chimerism status (p=0.29). In the with-MTX subgroup, CDC was in 44(52.4%) and MDC in 7(70%) participants. In the without-MTX subgroup, CDC was in 40(47.6%) and MDC in 3(30%). The third group of GVHD prophylaxis, with-steroids vs without-steroids, was also independent of donor chimerism status (p=0.16). In the with-steroids subgroup, CDC was in 14(16.7%) participants and none had MDC. In the without-steroids subgroup, 70(83%) of the participants had CDC and 10(100%) had MDC status.

In BMFS participants, median OS was 40months (range: 3-63 months). Median OS was 38 months (range: 3-63 months) and 48 months (range: 30-49 months) for the participants who had CDC and MDC, respectively. In BTM participants, median OS was 36 months (range: 2-54 months). The median OS in participants with CDC was 37 months (range: 2-54 months) and 46 months (range: 3-48 months) in those with MDC. OS of the cohort was 89(94.7%) with a mean survival of 153.06±3.45 months (confidence interval [CI]: 144.28-157.83). The OS in among those with BMFS was 52(95%) and among those with BTM it was 37(95%). The OS was associated with the chimerism status in the second month of post-transplant (p=0.02), shown in figure1 A and figure 2 A. In this cohort, participants with MDC status had an inferior OS of 8(80%) compared to the OS of 81(96.4%) in those with CDC. The hazard ratio for MDC at second month was 0.15 (p=0.04) (CI: 0.02-0.98).

In BMFS participants, median DFS was 38 months (range: 3-63 months); CDC 37 months (range 3-63 months), and MDC 48 months (range: 30-49 months). Median DFS in BTM cases was 35 months (range: 2-54 months); CDC 36 months (range: 2-54 months), and MDC 42 months (range: 13-48

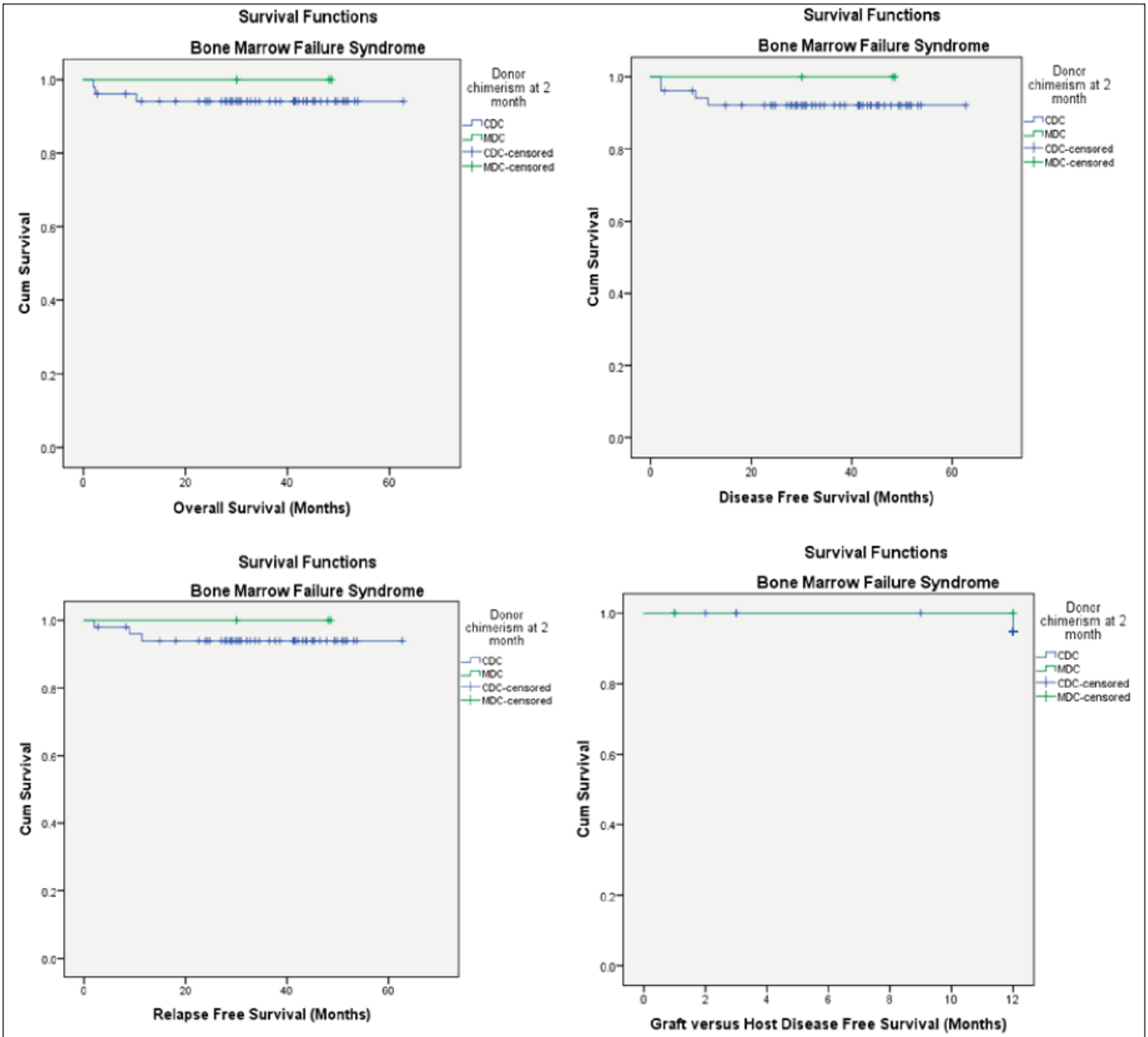

Figure-1: Association of donor chimerism with survival outcomes in post allo-HSCT patients of bone marrow failure syndrome (BMFS).
A) Overall survival graph of BMFS B) Disease free survival graph of BMFS D) Disease free survival graph of BTM C) Relapse free survival graph of BMFS survival graph of BMFS D) GVHD free survival graph of BMFS. [CDC: complete donor chimerism, MDC: Mixed donor chimerism.]

months). The DFS of the entire cohort was 87(92.6%); BMFS 51(92.7%) and BTM 36(92.3%). Early donor chimerism status was associated with DFS (p=0.01), shown in figure1 B and figure 2 B. Participants with MDC status had inferior DFS of 7(70%) compared to the DFS of 80(95.2%) in those with CDC status at second month post-transplant. The hazard ratio for MDC was low (p=0.02) (CI: 0.032-0.82).

The median RFS in BMFS participants was 37 months (range: 3-63 months); CDC 38 months (range: 3-63 months) and MDC 48 months (range: 30-49 months). Median RFS in BTM participants was 36 months (range: 2-54 months); CDC 36 months (range: 2-54 months), BTM 38 months (range: 2-48 months). RFS of the cohort was 89(94.6%) which was independent of chimerism status at 2 months, adjusted for underline diagnosis (p=0.06), shown in figure1 C and figure 2 C. RFS for BMFS was 52(94.4%) and for BTM it was 37(94.7%). In BMFS participants, relapse was found in 3(6.1%) participants with CDC, and 2(40%) BTM participants with MDC. Participants with MDC at second month had an inferior RFS of 7(77.8%) compared to the RFS

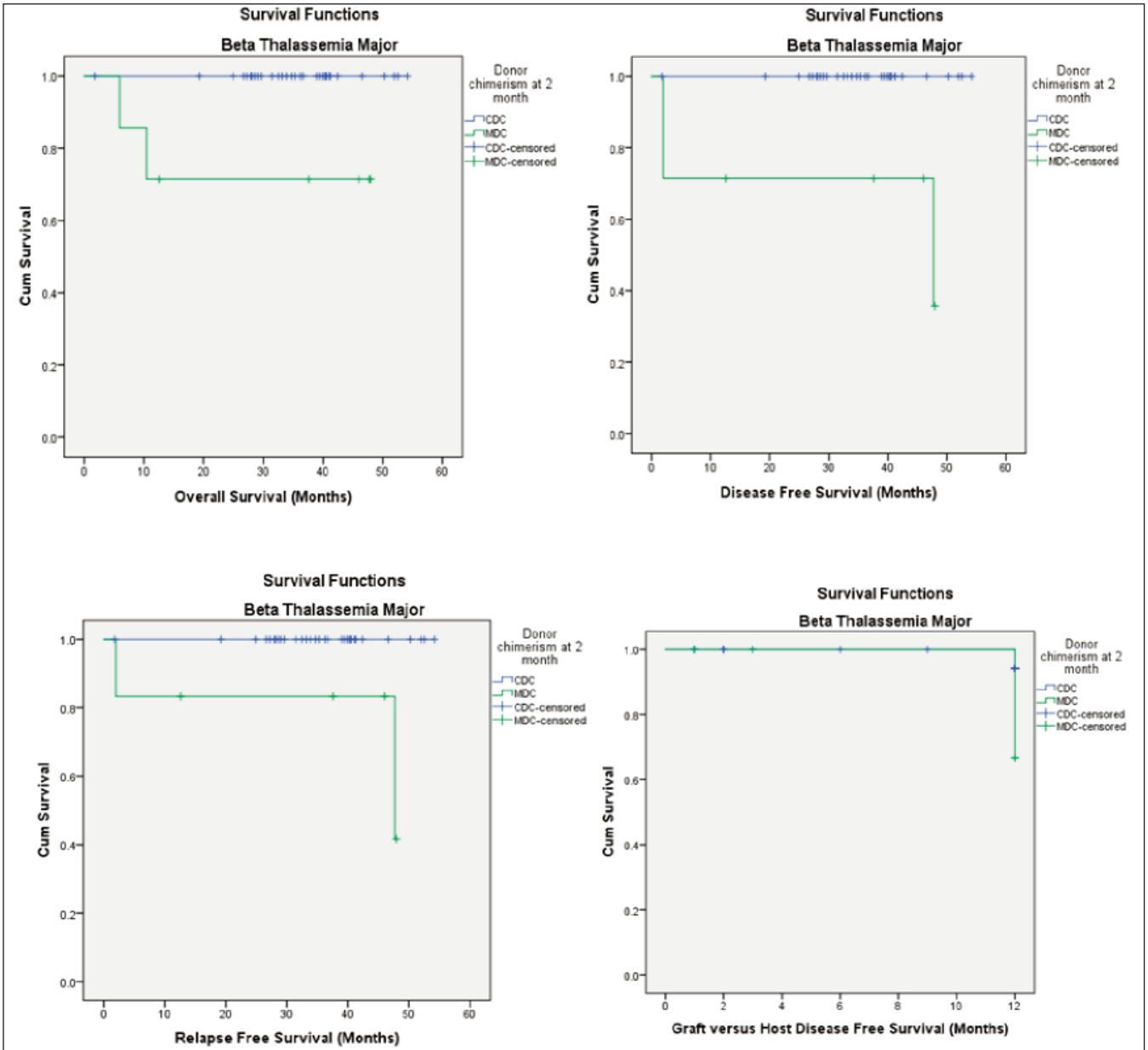

Figure-2: Association of donor chimerism with survival outcomes in post allo-HSCT patients of Beta thalassaemia Major (BTM).
A) Overall survival graph of BTM B) Disease free survival graph of BTM C) Relapse free survival graph of BTM D) GVHD free survival graph of BTM.
[*CDC: complete donor chimerism, MDC: Mixed donor chimerism.*]

of 80(96.4%) in CDC participants. Among the participants, 2(1.8%) died before relapse, and were thus excluded from RFS analysis.

One year GVHD-free survival of the cohort was 89(95.3%) with a median of 12 months (range: 1-12 months) which was not associated with chimerism status at 2 months (p=0.29), shown in figure1 D and figure 2 D. GVHD-free survival for BMFS was 52(96.1%) and for BTM it was 37(94.3%). GVHD-free survival was inferior with MCD 7(87.5%) compared to CDC 77(96.3%). GVHD at second month, in BTM group was found in 8(20.5%) participants; 7(21.9.3%) with CDC and 1(14.3%) with MDC status. At second month of post-transplant, GVHD in BMFS participants was found in 2(3.9%) participants with CDC. The occurrence of GVHD in participants of BTM was 21(50%) and participants of BMFS were 20(31.2%). These participants had GVHD of skin, eye, gut, and lung.

Overall, 3(4.6%) BMFS participants with MDC at second month post-transplant converted to CDC afterward. Also, 1(1.5%) patient remained in the MDC state without

transfusion. Among the BTM participants, 3(7.1%) out of 7(16.6%) died due to post-transplant complications, and 4(9.5%) were transfusion-dependent and were recommended for the second transplant.

Discussion

Chimerism analysis in post-allo-HSCT patients is a routine test to assess and monitor the graft status.[1] In addition, it also predicts impending relapse/graft failure in post-allo-HSCT patients. Previously, chimerism assessment was considered a surrogate marker for measurable residual disease in those diseases that do not have a molecular marker to assess the disease status. Now, chimerism status is also considered a post-allo-HSCT prognostic marker.[1]

In the current study, chimerism status at second month post-transplant was found independent of conditioning reagents and GVHD prophylaxis. The purpose of conditioning is to ablate the patients' bone marrow to eradicate the malfunctioning haematopoietic cells. Secondly, it also provides space or stem cell niches for the new stem cells of the donor. Thirdly, it provides enough immunosuppression that prevents rejection and GVHD. In aplastic anaemia and BTM, the effect of the conditioning regimen is myeloablative. However, it happens that the same conditioning regimen resulted in CDC status in one patient and in MDC in another patient. It may be due to the conditioning reagents which also change the bone marrow environment of patients.[17]

The chimerism status of post-allo-HSCT patients was independent of the GVHD prophylaxis administered. It is a routine practice that GVHD prophylaxis is used to prevent GVHD which continues in the post-allo-HSCT period till a specific time. In BTM, the immunosuppressant continues for three months before tapering starts in the absence of GVHD incidence and stops at the sixth month. In the case of aplastic anaemia, the immunosuppressant continues till the ninth month post-allo-HSCT before tapering starts in the absence of GVHD incidence and halts at the 15th month. As in GVHD, the donor T cells attack the patient's soft tissues and destroy the patient's residual haematopoietic cells1. In the current study, GVHD was present in 1(10%) participant with MDC, and 9(10.8%) participants with CDC in the presence of GVHD prophylaxis. Hence, GVHD and GVHD prophylaxis had no association with donor chimerism status.

The OS in the current study was 94.7% which is better than the OS reported earlier.[18-20] OS in participants with MCD was inferior to the participants with CDC, which is the ideal outcome in post-allo-HSCT patients, as it is assumed that CDC patients would remain with a good prognosis.

DFS is another desired outcome of post-allo-HSCT patients. In the current study, DFS was 92.3% which was less than the OS. DFS was found to be better than some other studies on nonmalignant diseases.[21,22] DFS is associated with early chimerism status, and DFS with CDC was superior (100%) to the DFS with MDC (57%) in BTM participants. However, DFS was 92.3% in CDC and 100% in MDC participants of BMFS.

In the curremt study, RFS was 94.6% which is better than the RFS in haematological diseases reported earlier.[22] RFS was not associated with early chimerism status. It is commonly known that MDC status predicts impending relapse or graft failure.[23] It seldom happens in nonmalignant disease that patients achieve partial remission with stable MDC status. It also occurs rarely that patients had complete donor chimerism as well as transfusion dependency. Therefore, such post-allo-HSCT patients are considered as having poor graft function, and require a second transplant.[1]

In the current study, one-year GVHD0free survival in BTM and BMFS was 95% which is comparable to an earlier report[24] and it was independent of chimerism status. In the management of post-allo-HSCT participants, at 1-year post-transplant, immunosuppressants are almost stopped or are put on tapering. According to studies, chimerism status has a relation with GVHD.[4,10] In other studies, the rationale for chimerism status not being associated with GVHD was that participants were taking immunosuppressants.[23,25]

In the past, those patients who had increased MDC along with transfusion dependency were recommended for the second transplant with the same donor or another donor. In recent years, such patients are either treated with donor lymphocyte infusion or managed by tapering/withdrawal of immunosuppressants.[2,3] In the study, 4 participants with increased MDC chimerism and transfusion-dependent were offered the second transplant, but all of them refused to take the offer. One of these participants did not transfuse and off all treatment himself. After two years of transplant, he revisited the hospital and had complete donor chimerism with chronic skin and eye GVHD. This may be due to the effect of immunosuppressant withdrawal.

The limitation of the current study was that it could not quantify the percentage of MDC status due to lack of funds.

Conclusion

Patients with early MDC status were found to be with inferior OS and DFS compared to those with CDC status. This was confirmed by survival outcomes.

Disclaimer: The text is based on a Ph.D thesis.

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References

1. Carreras E, Dufour C, Mohty M, Kröger N. The EBMT handbook: hematopoietic stem cell transplantation and cellular therapies. Cham CH: Springer, 2019.
2. Jiang Y, Wan L, Qin Y, Wang X, Yan S, Xie K, et al. Donor Chimerism of B Cells and Nature Killer Cells Provides Useful Information to Predict Hematologic Relapse following Allogeneic Hematopoietic Stem Cell Transplantation. PLoS One. 2015; 10:e0133671.
3. Clark JR, Scott SD, Jack AL, Lee H, Mason J, Carter GI, et al. Monitoring of chimerism following allogeneic hematopoietic stem cell transplantation (HSCT): technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. Br J Haematol. 2015; 168:26-37.
4. Stikvoort A, Gertow J, Sundin M, Remberger M, Mattsson J, Uhlin M. Chimerism patterns of long-term stable mixed chimeras post-hematopoietic stem cell transplantation in patients with nonmalignant diseases: follow-up of long-term stable mixed chimerism patients. Biol Blood Marrow Transplant. 2013; 19:838-44.
5. Mao P, Zhu Z, Wang H, Wang S, Mo W, Ying Y, et al. Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia. Eur J Haematol. 2005; 75:430-5.
6. Grimaldi F, Potter V, Perez-Abellan P, Veluchamy JP, Atif M, Grain R, et al. Mixed T Cell Chimerism After Allogeneic Hematopoietic Stem Cell Transplantation for Severe Aplastic Anemia Using an Alemtuzumab-Containing Regimen Is Shaped by Persistence of Recipient CD8 T Cells. Biol Blood Marrow Transplant. 2017; 23:293-9.
7. Yoshimi A, Bader P, Matthes-Martin S, Starý J, Sedlacek P, Duffner U, et al. Donor leukocyte infusion after hematopoietic stem cell transplantation in patients with juvenile myelomonocytic leukemia. Leukemia. 2005; 19:971-7.
8. Horn B, Soni S, Khan S, Petrovic A, Breslin N, Cowan M, et al. Feasibility study of preemptive withdrawal of immunosuppression based on chimerism testing in children undergoing myeloablative allogeneic transplantation for hematologic malignancies. Bone Marrow Transplant. 2009; 43:469-76.
9. Horn B, Petrovic A, Wahlstrom J, Dvorak CC, Kong D, Hwang J, et al. Chimerism-based pre-emptive immunotherapy with fast withdrawal of immunosuppression and donor lymphocyte infusions after allogeneic stem cell transplantation for pediatric hematologic malignancies. Biol Blood Marrow Transplant. 2015; 21:729-37.
10. Frugnoli I, Cappelli B, Chiesa R, Biral E, Noè A, Evangelio C, et al. Escalating doses of donor lymphocytes for incipient graft rejection following SCT for thalassemia. Bone Marrow Transplant. 2010; 45:1047-51.
11. Sairafi D, Remberger M, Uhlin M, Ljungman P, Ringdén O, Mattsson J. Leukemia lineage-specific chimerism analysis and molecular monitoring improve outcome of donor lymphocyte infusions. Biol Blood Marrow Transplant. 2010; 16:1728-37.
12. Israel GD. Determining sample size. Fact sheet PEOD-6. Gainesville, FL: University of Florida, 1992.
13. Ahmed S. Manual of PCR in Diagnostic Pathology. Pakistan: SAAD PrintoPak, 2013.
14. Dignan FL, Clark A, Amrolia P, Cornish J, Jackson G, Mahendra P, et al. D iagnosis and management of acute graft-versus-host disease. Br J Haematol. 2012; 158:30-45.
15. Vigorito AC, Campregher PV, Storer BE, Carpenter PA, Moravec CK, Kiem HP, et al. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood. 2009; 114:702-8.
16. Iacobelli S. Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant. 2013; 48:S1-37.
17. Kim CH, Wu W, Wysoczynski M, Abdel-Latif A, Sunkara M, Morris A, et al. Conditioning for hematopoietic transplantation activates the complement cascade and induces a proteolytic environment in bone marrow: a novel role for bioactive lipids and soluble C5b-C9 as homing factors. Leukemia. 2012; 26:106-16.
18. Shin SH, Park SS, Park S, Jeon YW, Yoon JH, Yahng SA, et al. Non-myeloablative matched sibling stem cell transplantation with the optional reinforced stem cell infusion for patients with hemoglobinopathies. Eur J Haematol. 2020; 105:387-98.
19. Ladis V, Chouliaras G, Berdoukas V, Chatziliami A, Fragodimitri C, Karabatsos F, et al. Survival in a large cohort of Greek patients with transfusion-dependent beta thalassaemia and mortality ratios compared to the general population. Eur J Haematol. 2011; 86:332-8.
20. D'Souza A, Lee S, Zhu X, Pasquini M. Current Use and Trends in Hematopoietic Cell Transplantation in the United States. Biol Blood Marrow Transplant. 2017; 23:1417-21.
21. Hashmi KU, Khan B, Ahmed P, Hussain I, Rasul S, Hanif E, et al. Allogeneic bone marrow transplantation in beta-thalassaemia--single centre study. J Pak Med Assoc. 2004; 54:499-503.
22. Alimoghaddam K, Ghaffari H, Foroughi F, Chardouli B, Sanaat Z, Bahar B, et al. Effects of chimerism on graft-versus-host disease, disease recurrence, and survival after HLA-identical marrow transplantation in Iran. Arch Iran Med. 2006; 9:99-103.
23. Mattsson J, Uzunel M, Remberger M, Ringdén O. T cell mixed chimerism is significantly correlated to a decreased risk of acute graft-versus-host disease after allogeneic stem cell transplantation. Transplantation. 2001; 71:433-9.
24. Bartelink IH, Lalmohamed A, van Reij EM, Dvorak CC, Savic RM, Zwaveling J, et al. Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. Lancet Haematol. 2016; 3:526-36.
25. Jiang Y, Wan LP, Qin YW, Wang XR, Yan SK, Xie KC, et al. Chimerism status is correlated to acute graft-versus-host disease after allogeneic stem cell transplantation. Int J Hematol. 2014; 99:323-8.