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Is mixed chimerism post allogeneic hematopoietic stem cell transplantation in pediatric acute lymphoid leukemia a prognostic factor for relapse ?

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ORIGINAL RESEARCH REPORT

Is Mixed Chimerism Post-allogeneic Hematopoietic Stem Cell Transplantation in Pediatric Acute Lymphoid Leukemia a Prognostic Factor for Relapse?

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Abstract

Hematopoietic stem cell transplantation (HSCT) has been considered curative for children with high-risk acute leukemia (ALL), offering better survival. Short tandem repeat has been used as a marker of chimerism status after HSCT. The appearance of recipient cells >1% post-allogeneic stem cell transplant is defined as mixed chimerism (MC). Chimeric studies post-HSCT are dynamic. This study aimed to investigate the significance of recipient cells in post-HSCT pediatric ALL patients as a predictor of relapse of their primary disease. The rate of MC was 51.4% (19 out of 37 recipients). It was 48.6% (n = 18) during Day+100 and 12.9% (4 out of 31 recipients) during post-Day+100 follow-up until two years. No significant association was noted between MC and all grade overall acute graft-versus-host disease. A mortality rate of 35.1% (n = 13) and a median follow-up of 56.9 months (95% CI: 39.7−74.2) were observed for all but four (16.7%) of the survivors in remission. Regarding causes of death, transplant-related mortality was recorded in only 2 of 13 expired patients (15.4%); both succumbed to sepsis. No significant association was found between MC and primary causes of death. The cumulative probability of five-year overall survival and event-free survival was not found to be statistically significantly different for MC (≤1.0% vs. > 1.0%). In conclusion, our data did not show MC testing alone as an effective prognostic marker for detecting relapse; molecular and flow cytometric analyses should be considered in children with ALL post-HSCT for monitoring relapse.

Keywords: Mixed chimerism, Stem cell transplantation, Pediatric, Acute lymphoid leukemia, Outcome

1. Introduction

Modern chemotherapy regimens treating acute lymphoblastic leukemia (ALL) result in remission for most patients. Hematopoietic stem cell transplantation (HSCT) is a possible curative option for the 20% of children who relapse [1]. The therapeutic effect of allogeneic (allo-) HSCT is attributed to the graft versus leukemia (GVL) effect produced by the engrafted cells. Factors responsible for relapse include conditioning regimens, graft source, cell dose, and functional effectiveness of the engrafted cells. Relapse after transplant can lead to potential morbidity and mortality in such patients [2].

Complete donor-derived hematopoiesis has been considered essential for sustained engraftment and relapse prevention in children. Hence, chimerism analysis is an important tool for engraftment surveillance that can identify impending graft rejection [3]. Several studies on the relationship between mixed chimerism (MC) and disease relapse revealed inconsistent results. Some studies proposed that MC at different times suggested imminent disease relapse [4,5], whereas other studies did not show this significant correlation [6−8].

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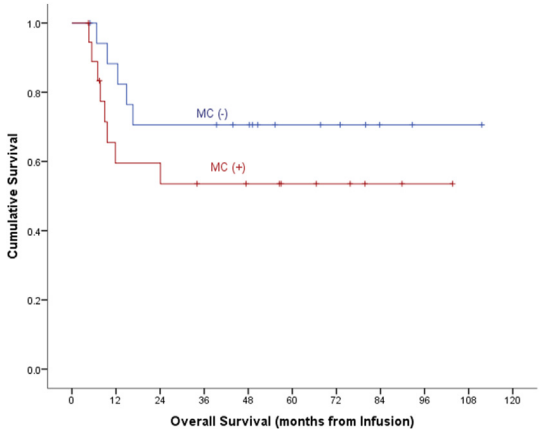

Fig. 1. Overall survival by MC.

The current gold standard method of monitoring chimerism is short tandem repeat (STR) polymerase chain reaction (PCR; STR-PCR), which determines the chimera and establishes the percentage of both donor and recipient cells [9,10]. Thus, the persistence and emergence of recipient cells after allo-HSCT can indicate the presence of malignant cells [11]. However, the heterogeneity in this technique varies among different centers.

In our retrospective review, the dynamics of MC in children receiving an allo-HSCT were analyzed to diagnose refractory or high-risk ALL. This study also reviewed the relationship between MC, clinical outcomes, and event-free survival (EFS).

2. Patients and methods

2.1. Study design

Forty-six transplant naïve pediatric patients (age at transplant <14 years) with ALL who underwent consecutive allogeneic HSCT from 2012 to 2017 at our institution were enrolled in this retrospective study. Thirty-seven (n = 37) were transplant naïve and lived beyond Day+120. Data on the presence of the recipient's DNA in this subgroup of patients was analyzed in the backdrop of clinical, transplant-related parameters, including donor human leukocyte antigens, post-transplant chimerism, engraftment, incidence of graft-versus-host-disease (GvHD), infectious- and non-infectious toxicity, and outcomes to investigate its significance as a predictor of relapse of primary disease. All parents and guardians signed institutional review board–approved parental informed consent before the transplant.

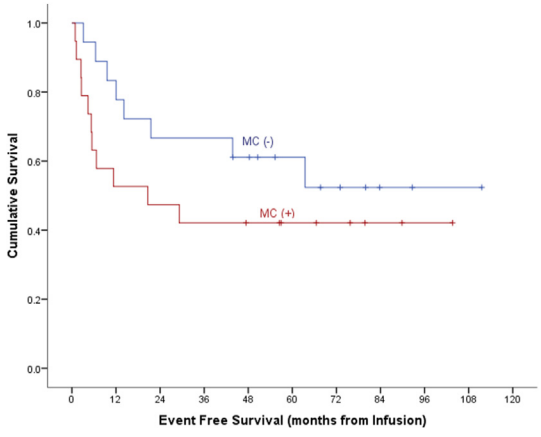

Fig. 2. Event-free survival by MC.

Abbreviations

ANC	Absolute neutrophil count
HSCT	Hematopoietic Stem Cell Transplantation
PBSC	Peripheral Blood Stem Cells
GvHD	Graft versus host disease

2.2. Transplant procedure

Patients were hospitalized until neutrophil recovery in single rooms with high-efficiency particulate air filtration with positive pressure. Patients received acyclovir prophylaxis if they were seropositive for herpes simplex virus or cytomegalovirus (CMV). Pneumocystis prophylaxis was administered after engraftment for one year or more, depending on the immune reconstitution status of the patients. Broad-spectrum intravenous antibacterial, antifungal, and antiviral medications were administered for fevers and infections as indicated. Patients received granulocyte colony-stimulating factor (5 μg/kg per day) subcutaneously until neutrophil recovery. CMV reactivation was monitored weekly following transplant and preemptively treated with ganciclovir or foscarnet, depending on the engraftment status. Different agents were used as GvHD prophylaxes. Cyclosporine (CSA) and methotrexate (MTX) were used in 24 cases, while mycophenolate mofetil and CSA were used in 6 cases. CSA alone was used for four patients, in combination with steroids for two, and only one patient was given MTX alone.

2.3. Chimerism measurement

Chimerism monitoring was conducted using kits to amplify a panel of informative STR loci/microsatellite regions via PCR. Donor contribution was calculated by integrating donor-specific and

recipient-specific informative STR peak areas for detecting chimerism status. Peripheral blood (PB) samples were collected and analyzed duringengraftmentor on day 30.

Lymphoid cell MC was selected because several studies have shown that T cell MC is associated with relapse in several settings, including myeloid and lymphoid malignancies using both myeloablative and non-myeloablative regimens [12,13]. Thereafter, chimerism monitoring was attempted every two to three months for one year after transplantation. A quantitative recipient-specific STR-PCR signal $\leq$1% on day 30 was defined as complete donor chimerism. MC was defined as the presence of $\geq$1.0% recipient cells in the lymphoid cell lineage from PB [14].

2.4. End-point definitions

Time to neutrophil recovery was the first of three consecutive days on which the absolute neutrophil count (ANC) was $\geq 0.5 \times 10^9$/L. Primary graft failure was defined as failure to achieve an ANC of 0.5×10^9/L by day 42, and secondary graft failure as an ANC $<0.5 \times 10^9$/L for three consecutive days or 0% donor chimerism by PCR. Time to platelet recovery was the first of three consecutive days on which the platelet count was $>20 \times 10^9$/L without transfusions for seven days before the first measurement. GvHD was graded using standard criteria. Relapse of primary disease and death from any cause was considered an event. Overall survival (OS) was taken from the time of infusion to patient expiry or the last contact date, with death from any cause as an event.

2.5. Statistical analysis

All continuous data are presented as the median with minimum and maximum points. An Independent-sample Mann–Whitney U test was used to test the significance of difference among the continuous variables. Chi-square test or Fisher's Exact test were employed to test the significance of the association between categorical variables. Kaplan–Meier curves were drawn for survival analyses. IBM-SPSS for Windows (version 20.0) was used for statistical data analysis.

2.6. Ethical considerations

Before initiation, this study was submitted to the Institutional Review Board of King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. It was approved by the Research Advisory Committee through established procedures (Approval Number 2201265).

2.7. Ethical compliance with human/animal study

This study was conducted in compliance with the ethical standards of the responsible institution on human subjects and with the Helsinki Declaration.

3. Results

The median age of the cohort at HSCT was 8.8 years (range, 0.5–14.0 years); almost half of the recipients were females (n = 18, 48.6%). Patient characteristics and transplant-related parameters for the cohort are provided in Table 1. The median follow-up time was 56.9 ± 8.8 months (95% Confidence Interval: 49.7–74.2 months). All 37 patients were conditioned with a myeloablative chemotherapy regimen and received GvHD prophylaxis. Radiation was part of the conditioning regimen in 31 (83.8%) recipients. Bone marrow (BM) was the source of stem cells in 28 (75.7%) cases. The donor was a fully matched relative in 24 (64.9%) cases and a haploidentical family member in 3 (8.1%) cases. All recipients had normal renal and cardiac functions at the time of transplant.

3.1. Mixed lymphocyte quantitative chimerism

The rate of MC was 51.4% (19 out of 37 recipients). It was 48.6% (n = 18) during Day+100 and 12.9% (4 out of 31 recipients) during post-Day+100 follow-up until two years, where applicable.

3.2. Engraftment

ANC recovery was observed in all cases with a median time to recovery of 15 days (range, 13–38 days), and platelets recovery was observed in 35 recipients with a median recovery time of 30 days (range, 17–96 days); 26 (74.3%) achieved this within 42 days post-infusion. No statistically significant difference was found for median time to ANC recovery for patients transplanted with BM or PB compared to those transplanted with cord blood (CB; P = 0.086). However, the median time to platelet recovery was significantly lower in the BM/PB group than in the CB group (P = 0.005, Table 2). Our cohort median time to relapse of primary disease was 6.7 months (range, 0.9–63.5). No event of primary graft failure was observed, whereas the incidence of secondary graft failure was 5.4% (n = 2). Both patients who lost their graft were transplanted using CB and succumbed to their

Table 1. Patient characteristics and transplant-related parameters.

Parameters of interest, n (%)	Overall (n = 46)	Enrolled[c] (n = 37)
Gender		
Female	22 (47.8%)	18 (48.6%)
Male	24 (52.2%)	19 (51.4%)
Age at HCT		
median (range), years	7.6 (0.5–14.0)	8.8 (0.5–14.0)
Pre-infusion disease status		
CR-1	19 (44.2%)	16 (44.4%)
CR-2	22 (51.2%)	18 (50.0%)
CR-3	2 (4.7%)	2 (5.6%)
Source of Stem Cells		
Bone marrow	32 (69.6%)	28 (75.7%)
PBSC	2 (4.3%)	1 (2.7%)
Cord blood	12 (26.1%)	8 (21.8%)
TNC dose[a], median (range) for BM/PBSC	0.6 (0.1–103.1)	0.5 (0.2–84.8)
TNC dose[a], median (range) for CB	0.071 (0.03–0.4)	0.072 (0.03–0.38)
CD34[b], median (range) for BM/PBSC	5.4 (1.7–13.5)	5.4 (1.7–13.5)
CD34[b], median (range) for CB	0.2 (0.01–2.3)	0.19 (0.05–2.3)
Recipient weight (kg) at infusion, median (range)	20.0 (6.0–78.5)	21.9 (6.0–78.5)
Conditioning regimen		
Cytarabine, TBI	23 (50.0%)	20 (54.1%)
Cytarabine, TBI, ATG	11 (23.9%)	7 (18.9%)
Fludarabine, TBI	5 (10.9%)	4 (10.8%)
Busulfan, Cytarabine (±ATG)	4 (8.6%)	4 (10.8%)
Busulfan, Cytarabine, VP-16 (±ATG)	1 (2.2%)	1 (2.7%)
Busulfan, Fludarabine, Thiotepa	1 (2.2%)	–
Fludarabine, Cytarabine, ATG	1 (2.2%)	1 (2.7%)
Donor Gender (BM/PBSC only)		
Female	21 (61.8%)	17 (58.6%)
Male	13 (38.2%)	12 (41.4%)
Donor-Recipient gender matching		
Same gender	16 (47.1%)	14 (48.3%)
Female → male	10 (29.4%)	8 (27.6%)
Male → female	8 (23.5%)	7 (24.1%)
HLA Type		
BM/PBSC		
HLA identical siblings	23 (67.6%)	21 (72.4%)
HLA identical parent	4 (11.8%)	3 (10.3%)
Haploidentical family member	5 (14.7%)	4 (13.8%)
Related 1-AG mismatched donor	2 (5.9%)	1 (3.4%)
Cord Blood		
Related 1-AG mismatched donor	1 (8.3%)	1 (12.5%)
Unrelated HLA identical	1 (8.3%)	1 (12.5%)
Unrelated HLA 1- or 2-AG mismatch (CB)	10 (83.3%)	6 (75.0%)

[a] 10^9 per kg.
[b] 10^6 per kg.
[c] Lived beyond day120.

disease progression. These two patients comprised 15.4% of the overall mortality rate of 35.1% for our cohort (2 out of 13 deaths). No significant association was found between relapse of primary disease and source of stem cells (BM/PBSC: 15 of 29, 51.7% vs. CB: 4 of 8, 50.0%, P = 1.000).

3.3. Graft-versus-host disease

The cumulative incidence of all grade overall acute GvHD was 43.2% (n = 16), whereas grades I–II acute GvHD accounted for 29.7% (n = 11), and severe acute GvHD (grade III) was seen in five cases (13.5%). No significant association between MC and all grade overall acute GvHD was noted. Chronic GvHD was observed in 3 of 27 evaluable cases (11.1%); all were seen in skin and were limited in grade, and only one case was found positive for MC.

3.4. Infections and transplant-related toxicity

Regarding transplant-related morbidity during the first 100 days, 13 (35.1%) recipients had cytomegalovirus reactivation, which was managed successfully using ganciclovir and/or foscarnet (Table 2). Two (5.4%) of the recipients had hemorrhagic cystitis; none of our recipients had sinusoidal obstructive syndrome or interstitial pneumonia. There were 15 (40.5%) bacterial infections, 6 (16.2%) viral, and 0 fungal infections recorded in the cohort. None of our patients had transplant-associated thrombotic microangiopathy.

3.5. Survival, mortality, and causes of death

With a mortality rate of 35.1% (n = 13) and a median follow-up of 56.9 months (95% CI: 39.7–74.2), all but four (16.7%) of the survivors were in remission. The cumulative probability of five-year OS and EFS was not found to be statistically significantly different for MC (≤1.0% vs. > 1.0%, Figs. 1 and 2). No significant association was found between MC and primary disease relapse or mortality (Table 3). All mortalities (n = 13, 35.1%) were seen among relapsed cases; however, relapse of primary disease (n = 19, 51.4%) was found to be significantly associated with mortality ($P < 0.001$).

Regarding causes of death, transplant-related mortality was recorded in only 2 of 13 expired patients (15.4%); both succumbed to sepsis. No significant association was found between MC and primary causes of death.

Table 2. Transplant outcome and survival benefits by MC.

Outcome	All recipients (n = 37)
Days to ANC recovery, median (range), n	15 (13–38), 37
BM/PBSC (n = 29)	15 (13–25), 29
CB (n = 8)	26 (13–38), 8
Days to platelets recovery, median (range), n	30 (17–96), 35
BM/PBSC (n = 29)	26 (17–84), 29
CB (n = 8)	49.5 (32–96), 6
Acute GvHD (+)	16 (43.2%)
Overall Grade I–II	11 (68.8%)
Overall Grade III–IV	5 (31.3%)
Skin	12
Grade I–II	*12 (100.0%)*
Grade III–IV	*None*
Liver	None
Grade I–II	*None*
Grade III–IV	*None*
Gut	8
Grade I–II	*8 (100.0%)*
Grade III–IV	*None*
Infectious toxicity (during day+100)	
Bacterial	15 (40.5%)
Viral	6 (16.2%)
Fungal	None
Post-HSCT relapse of primary disease	19 (51.4%)
Time to relapse from infusion, months	6.7 (0.9–63.5)
Sites of relapse of primary disease	
Blood	13 (68.4%)
CNS	5 (26.3%)
Bone marrow	1 (5.3%)
Relapse rate by MC*	
MC (≤1.0%)	8 (42.1%)
MC (>1.0%)	11 (57.9%)
Mortality rate by MC throughout follow-up**	13 (35.1%)
MC (≤1.0%)	5 (38.5%)
MC (>1.0%)	8 (61.5%)
Cumulative probability of 5-year overall survival***	62.1% ± 8.3%
MC (≤1.0%)	70.6% ± 11.1%
MC (>1.0%)	53.6% ± 12.1%
Cumulative probability of 5-year event-free survival****	51.4% ± 8.2%
MC (≤1.0%)	61.1% ± 11.5%
MC (>1.0%)	42.1% ± 11.3%

IMC, Increasing mixed chimerism from infusion to ~2 years*P = 0.517 **P = 0.495, ***P = 0.186 ****P = 0.106.

4. Discussion

Chimerism testing aims to identify genomic regions with enough polymorphic diversity to distinguish donor versus recipient genetic origin in patient specimens. Most institutions perform chimerism analyses routinely for surveillance of engraftment after HSCT in pediatric patients with hematologic malignancies. Generally, PB analysis is preferred over BM analysis because of lineage-specific results. Based on these results, some institutions are utilizing chimerism studies for immunotherapy to rescue patients with MC [15,16]. Herein, for our pediatric ALL cohort, the trend of STR-PCR post-transplant was studied as the current recommended method for engraftment monitoring [17,18].

Chimerism in PB was used over BM, given that there is no difference between them [19]. We hypothesized that MC leads to graft rejection and an increased risk of relapse. The question of MC being indicative of an increased risk of relapse at any time point post-HSCT has been discussed controversially over the years [5,16,20,21]. Many retrospective studies have shown variable timelines between autologous cell detections and relapse. In our patient cohort, we observed that persistent MC or/and MC detection at any time point did not correlate with increased relapse rates.

Relapse is most commonly seen during the first two years post-HSCT. Our cohort median time to relapse of primary disease was early at 6.7 months. The duration of follow-up for chimerism monitoring should be compatible with the timeline of relapse post-HSCT [22]. There was no significant difference in the timing of the chimerism check and type of chimerism status in children transplanted for ALL, in line with other published studies [20,23]. MC can represent autologous recipient hematopoietic recovery, and this should not necessarily raise concerns for relapse in the setting of malignancy. Most institutions now agree that MC is a more dynamic and common state than previously thought [24].

Mountjoy et al. questioned the utility of continued early chimerism testing based on a retrospective

Table 3. Transplant outcome and survival by MC.

Outcome	Max. MC (until Day+100)		P value	Max. MC (Day+100-Last contact)		P value	Max. MC (Throughout F-up)		P value
	≤1.0%	>1.0%		≤1.0%	>1.0%		≤1.0%	>1.0%	
Primary disease	(n = 19)	(n = 18)	0.746	(n = 27)	(n = 4)	0.284	(n = 18)	(n = 19)	0.517
In remission	10 (52.6%)	8 (44.4%)		17 (63.0%)	1 (25.0%)		10 (55.6%)	8 (42.1%)	
Relapsed	9 (47.4%)	10 (55.6%)		10 (37.0%)	3 (75.0%)		8 (44.4%)	11 (57.9%)	
Survival status			0.737			0.560			0.495
Alive	13 (68.4%)	11 (61.1%)		20 (74.1%)	2 (50.0%)		13 (72.2%)	11 (57.9%)	
Expired	6 (31.6%)	7 (38.9%)		7 (25.9%)	2 (50.0%)		5 (27.8%)	8 (42.1%)	

analysis at their center showing that neither T cell nor myeloid cell chimerism at day 30 or 60 had a statistically significant impact on OS [25]. Analysis of total donor cell chimerism is compromised by the limited level of sensitivity, ranging between 1 and 5% for STR. Minimal residual (MRD) testing sensitivity is in the order of 0.01% [23,26]. There is limited comparative data on both being used simultaneously. A recent analysis by Pincez et al. suggested that BM monitoring with MRD is more effective for early detection of post-HSCT leukemia recurrence [27].

Low donor T cell chimerism in blood was shown by Mossallam et al. to be significantly associated with a reduced risk of chronic GvHD [28]. This was not observed in our limited number of patients. All our patients were transplanted using a myeloablative regimen, and it has been shown that conditioning regimens have no impact on the patient's chimerism status [20]. When ATG was used as a GvHD prophylaxis, engraftment with complete donor chimerism was observed later than that in patients without ATG. ATG was not used in our cohort, hence the significance of early and continuous T cell chimerism monitoring [28].

Limitations of our study include its retrospective design, small number of patients, heterogenous patient characteristics and transplants, and lack of frequent time points for chimerism analysis. Our data suggest that chimerism testing alone in pediatric patients post-HSCT for malignant diseases does not seem reliable for relapse prediction. Our institutional practice does not include post-HSCT MRD. Hence we do not have data for comparative purposes. Bader et al. showed that detection of MRD post-HSCT is cost-effective and indicative of impending leukemia relapse [29]. When concerned about relapse, molecular and flow cytometric analyses should be considered in children. We feel that chimerism testing alone is ineffective in detecting ALL relapse post-HSCT.

Financial disclosure or funding

This study did not receive any financial support from any funding agency.

Informed consent

Data of interest collected from the patients' medical records were secured as governed by the institutional policies on patient confidentiality and privacy. No informed consent was obtained since this was a retrospective study, and all data items collected were already documented in medical charts as part of the patient care and disease management documentation.

Author contributions

All authors certify that they have participated sufficiently in the intellectual content and data analysis. Each author has reviewed the final version of the manuscript and approved it for publication. Should the editors request the data upon which the study is based, the authors shall produce it.

Institutional review board approval

Before initiation, this study was submitted to the Institutional Review Board of King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. It was approved by the Research Advisory Committee through established procedures via Approval Number 2201265.

Ethical compliance with human/animal study

This study was conducted in compliance with the ethical standards of the responsible institution on human subjects and with the Helsinki Declaration.

Data availability

The data can be made available upon reasonable request to the corresponding author.

Conflict of interest

The authors declare no conflicts of interest.

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