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Original Article

Early achievement of full donor chimerism after allogeneic hematopoietic stem cell transplantation predicts lower relapse risk in patients with acute lymphoblastic leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) remains one of the most difficult-to-cure hematological malignancies. Allogeneic hematopoietic stem cell transplantation (HSCT) provides curative potential but a substantial proportion of patients eventually will relapse. It is unknown if there are any modifiable factors exists that could improve survival or predict relapse immediately after HSCT is unknown. The aim of this study was to explore whether achieving early (<30 days) full donor chimerism (FDC) could predict disease relapse after allogeneic HSCT in ALL patients. A second objective is to examine the impact of achieving early donor chimerism on survival.

Methods: This study retrospectively enrolled 55 ALL patients undergoing allogeneic HSCT during the 10-year period from 1999 to 2008. Analysis of short tandem repeats (STR) was used to determine donor chimerism, and was prospectively followed at the time of engraftment and on days 30. Patients with early treatment-related mortality (<30 days), without STR analysis, or who were lost to follow-up before FDC were excluded. Survival analyses were performed using Kaplan−Meier Methods. Cox proportional hazard analyses were performed for poor prognostic factors associated with overall survival (OS) and relapse-free survival (RFS).

Results: The general characteristics were comparable between patients with early donor chimerism (n = 31) and those with late donor chimerism (n = 24). Survival analyses showed patients with early FDC had both lower probability of relapse ($\chi^2 = 5.770$, $p = 0.022$) and longer RFS than those with late chimerism. The OS was not different according to the chimerism status on days 30. In the Cox proportional hazard analyses, early FDC is a significant factor predictive for longer RFS (HR = 0.264, $p = 0.010$).

Conclusion: Our results indicate that the achievement of early FDC within 30 days after allogenic HSCT can be used as a significant predictor of RFS. The results underscored the need to improve outcome in ALL patients with late FDC.

Keywords: Acute lymphoblastic leukemia; Chimerism; Hematopoietic stem cell transplantation; Survival

1. Introduction

Acute lymphoblastic leukemia (ALL) is one of the most difficult-to-cure hematological malignancies. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the mainstay of treatment for high risk patients. Post-HSCT routine chimerism analysis of peripheral blood or bone

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marrow samples has been established to determine donor/recipient hematopoiesis and guide possible prophylactic or salvage immunotherapy,[1] such as donor lymphocyte infusion (DLI). A substantial number of studies focusing on the relationship between mixed chimerism (MC) and disease relapse had revealed inconsistent results. Some studies proposed that mixed chimerism[2,3] at different times or variable chimerism kinetics[4–7] (loss of full donor chimerism [FDC] or increasing MC) suggested imminent disease relapse, whereas other studies did not show significant correlation.[8,9] Such results might vary depending on the immunological condition of different age groups and of disease status during treatment. For example, leukemia patients without complete remission might be exposed to more chemotherapy causing greater immune suppression.[10] Moreover, a myelo-ablative conditioning (MAC) regimen would impose greater immunosuppressive effects on patients than those with reduced-intensity regimens, thereby resulting in different chimerism kinetics post-transplant. The heterogeneity in the analysis and method of chimerism,[7,11,12] and the different leukocyte subsets[5,6,10,13] measured for chimerism monitoring, might also contribute to the controversy. Several cohort studies of pediatric patients with ALL demonstrated that MC predict lower relapse-free survival,[2,6,7,11] whereas few articles on adult ALL patients were reported.[14] In this study, we aim to assess the relationship between clinical outcome and the time to achieve early FDC in ALL patients.

2. Methods

2.1. Patients

From January 1, 1999 to January 1, 2009, a total of 66 consecutive ALL patients who had undergone allo-HSCT were enrolled in this study. Patients with early treatment-related mortality (≤30 days), no analysis for short tandem repeat (STR), or lost to follow-up were excluded. Fifty-five patients were eligible for analysis, including 19 young than age 18. This study was conducted according to the Declaration of Helsinki, and was approved by the Institutional Review Board of Taipei Veterans General Hospital in Taiwan (VGH IRB no.: 201703002BC). Informed written consent was waived by the approving IRB. In addition, patient record/information was anonymized and de-identified before analysis.

2.2. Transplantation details and post-HSCT care

The donors were categorized as sibling donors (SD) or unrelated donors (UD). MAC preparations include busulfan (4 mg/kg/day for 4 days) combined with cyclophosphamide (60 mg/kg/day for 2 days), or total body irradiation (TBI) of 1200 cGy combined with cyclophosphamide (60 mg/kg/day for 2 days). To prevent acute graft–versus-host disease (GVHD), all of then patients received intravenous cyclosporine at a dose of 3 mg/kg/day in two split administration, with adjusted trough plasma level of 100–250 ug/L. Generally, the cyclosporine dose was de-escalated starting 2 months after HSCT over a 3-month period. Rabbit anti-thymocyte globulin (ATG) was administered at a total dose of 4–6 mg/kg on day −2 and day −1 for cases transplanted with unrelated donors. Short-term methotrexate was infused on the first (15 mg/m^2), third, sixth, and eleventh (10 mg/m^2, respectively) days after transplantation. The severity of acute GVHD was graded according to the system of Glucksberg and Thomas[15] and the severity of chronic GVHD was determined using the revised Seattle classification.[16] Donor lymphocyte infusion (DLI) was adopted for high risk patients and for cases with persistent chimera after HSCT. The CD3 cells were infused at an escalating dose, starting from 1×10^5/kg to 5×10^6/kg and were infused for at least a 2-week interval.

Table 1
Clinical characteristics of study population.

Patient characteristics	Cohort, n = 55		χ^2 (p)
	Time to FDC ≤30 days (n = 31)	Time to FDC >30 days (n = 24)	
Age at diagnosis			0.545 (0.571)
>16 years	19	17	
<16 years	12	7	
Sex			0.181 (0.787)
Male	16	11	
Female	15	13	
Diagnosis			0.812 (0.422)
B-ALL	13	13	
Non-B ALL[a]	18	11	
Donor type			1.087 (0.414)
SD	16	9	
UD	15	15	
Graft source			1.336 (0.750)
Bone marrow	10	7	
Peripheral blood	21	17	
CD34 cell dose			0.753 (0.523)
Cell dose ≤ 4×10^6	9	9	
Cell dose > 4×10^6	14	8	
Unavailable data	8	7	
Disease status before HSCT			0.684 (0.575)
Complete remission (CR)	30	22	
CR1	22	16	
CR2	8	6	
None	1	2	
Conditioning Regimen			0.622 (0.482)
Chemotherapy-based[b]	4	5	
TBI-based (12Gy)	27	19	
GVHD prophylaxis			0.812 (0.422)
CsA plus MTX	31	24	
Combination with ATG	13	13	
Grading of acute GVHD			1.408 (0.370)
Grade 0-1	20	19	
Grade 2-4	11	5	
Extent of chronic GVHD			0.345 (0.765)
None to limited	21	18	
Extensive	10	6	
Relapse			5.77 (0.022)
Yes	6	12	
No	25	12	

CsA = cyclosporin A; MTX = methotrexate.
[a] Including 14 patients with T-cell type, 13 with unknown lineage, 1 with mixed lineage, and 1 with bi-phenotype ALL.
[b] Mostly Busulfan-Cyclophosphamide regimen (8/9).

Patient characteristics, graft source and remission status before allo-HSCT are detailed in Table 1. The principal protocols, such as GVHD prevention, and post-transplant care for allo-HSCT recipients in Taipei Veterans General Hospital have been well-defined.[17] Treatment of acute and chronic GVHD was in general consistent with the guidelines.[18,19] Systemic steroid, usually methylprednisolone at doses of 1−2 mg/kg/day was the mainstay treatment for GVHD. Cyclosporine or other immunosuppressive agents such as mycophenolate mofetil were individualized at discretions of the attending physician when persistent/worsening GVHD was noted despite steroid treatment.

2.3. Chimerism measurement

Chimerism monitoring was conducted using the AmpFISTR Profiler Plus Kit (Applied Biosystems, Foster City, CA, USA) to amplify a panel of nine informative STR loci/microsatellite regions and an additional gender-determining marker Amelogenin via the polymerase chain reaction (PCR) method. All of the PCR products were separated on the ABI Prism 310 Genetic analyzer (Applied Biosystems) and the fragment sizes were analyzed using GeneScan software. Recipient-specific alleles were first chosen as informative STR foci as appropriate. Donor contribution was calculated by integrating donor-specific and recipient-specific informative STR peak areas with estimated sensitivity between 1% and 6% for detecting mixed chimerism.[20] Either peripheral blood or bone marrow samples were collected and analyzed at the time of engraftment and on days 30. Thereafter, chimerism monitoring was performed every one to two months for one year after transplantation. A quantitative recipient-specific STR-PCR signal less than 1% on days 30 was defined as early FDC, otherwise it was MC.

2.4. Statistical analysis

The probability of survival was estimated using the Kaplan−Meier method. Pearson's exact test was used to compare the categorical variables between the patients who attained FDC by days 30 or not. Univariate and multivariate Cox proportional models were designed to investigate the impact of early FDC on the RFS, considering the competing risk of relapse. OS was defined as the duration from transplantation to death or the last follow-up. Any post-transplant death resulting from infection, GVHD, or graft failure was deemed transplant-related mortality (TRM). Independent variables with $p < 0.1$ in the univariate model were included in the multivariable analysis. All of the statistical significances were set at $p < 0.05$.

3. Results

Of the 55 patients (median age: 22 years; range 0.4−57.3) studied, 31 had early FDC, and 24 MC. The majority of the patients were in complete remission (CR1 69%, CR2 25.4%) prior to transplant. The median follow-up time after HSCT was 477 days (range: 34−3459 days). All of the patients attained hematological CR and FDC in variable time periods (days 13−145, median time to FDC: days 30) (Fig. 1). The timing of FDC had a significant association with the probability of relapse ($\chi^2 = 5.770$, $p = 0.022$) (Table 1).

Relapses occurred in 18 patients (33%), including six in the group attaining early FDC (median: 15 days), and 12 in the group with late FDC (median: 54 days). For the relapsed patients, the time from FDC to relapse did not differ significantly by Kaplan−Meier plotting (median: 157 days vs. 131 days, $p = 0.616$ for the early FDC group versus the MC group). All of the relapses developed before the twenty-forth month for those with early FDC, compared to the tenth month (10/12) for those with MC by days 30. In the relapsed patients, four of 12 with MC by days 30 received rescue DLI compared to none of six with early FDC. A total of 28 patients (50.9%) died: 11 died of disease relapse or progression and 17 died of transplant-related mortality (TRM). TRM did not differ (HR 0.987, $p = 0.9$) by chimerism status on days 30. More patients died within 30 days after relapse in the early FDC group (2/6) than in the MC group (1/12). Analyzing the impact of early FDC on survival outcome, a significant difference was noted for 3-year RFS (median RFS, 486 versus 252 days, $p = 0.006$) (Fig. 2A) but not for 3-year OS (median OS 559 versus 427 days, $p = 0.132$) (Fig. 2B). The patients transplanted at first remission had a superior 3-year RFS in both univariate (HR = 0.342, $p = 0.026$) (Table 2) and multivariate analysis than those at second or non-remission (HR = 0.327, $p = 0.024$) (Table 3). A Cox regression model demonstrated that early FDC was associated with longer RFS (HR = 0.264, 95% CI 0.096−0.725, $p = 0.010$) after adjusting pre-transplant remission status as a confounding factor for relapse (Table 3).

4. Discussion

Clone-specific markers for minimal residual disease (MRD) monitoring had become standard practice in the treatment course of ALL; however, they are more frequently applied in

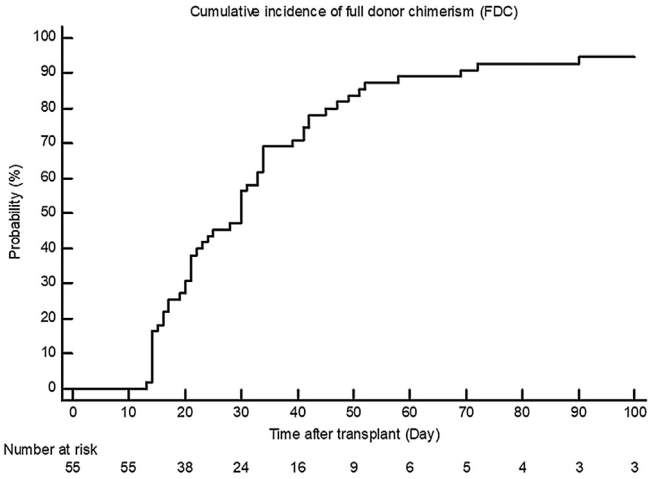

Fig. 1. **Cumulative incidence of FDC**. median time to FDC: days 30; all patients attained FDC by days 13−145.

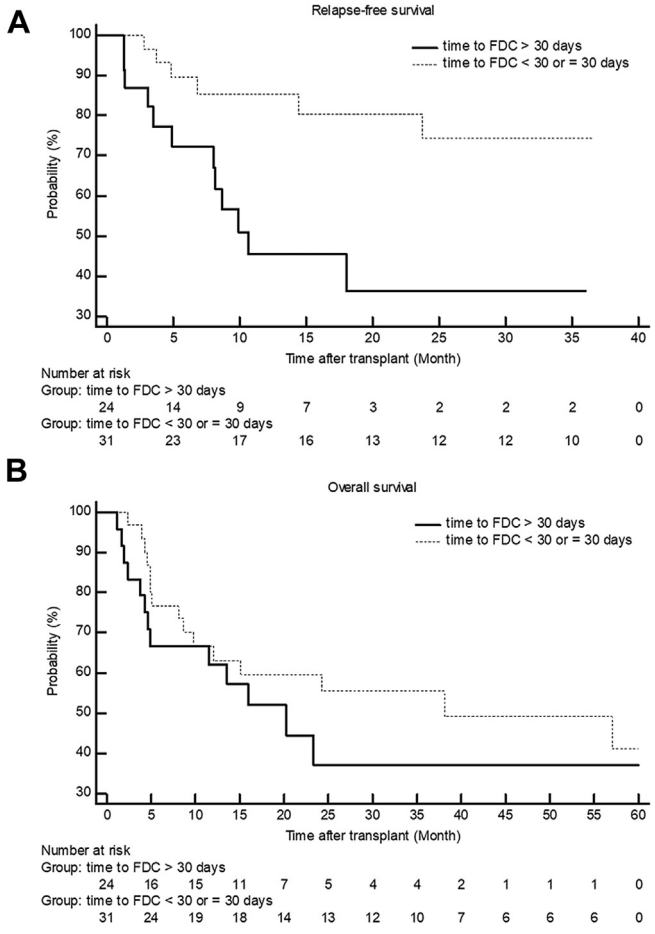

Fig. 2. (**A**). **RFS for patients achieving early FDC or not**. A significant difference for 3-year RFS (median RFS, 486 versus 252 days, $p = 0.006$). (**B**). **OS for patients achieving early FDC or not**. Non-significant difference in 3-year OS (median OS 559 versus 427 days, $p = 0.372$).

pediatric patients[21,22] than adults. Although Spinelli et al.[23] reported that MRD negativity by days 100 after allo-HSCT was significantly associated with a lower 3-year relapse incidence for adult patients with ALL, further validation in larger adult cohort should be conducted. Furthermore, MRD monitoring is not always applicable when lacking suitable markers. Terwey et al. had also reported three of seven MRD-positive relapse cases had antecedent MC signals prior to MRD detection,[14] suggesting the value of chimerism analysis as a surrogate marker indicating imminent relapse in real world practice.

Previous studies had reported poor RFS in child ALL patients with increasing MC post-transplant.[6,7] Herein, we provided a single center experience demonstrating how early FDC affect outcome in a cohort enrolling mainly adult ALL patients, who received TBI-based MAC followed by unmanipulated stem cell infusion. This study revealed that early FDC predicted a significantly better RFS but not with OS.

Three additional studies had also addressed the significance of days 30 whole blood chimerism in predicting disease recurrence. In data reported by Reshef et al.,[24] every 1% increase in whole blood chimerism on days 30 corresponded to a 10% decrease in relapse risk. In a study conducted by Koreth et al.,[25] mixed total donor cell chimerism less than 90% on day 30 post-HSCT was predictive of increased risk of relapse (HR 1.66, $p < 0.0001$). A. Lassaletta et al.[2] showed that pediatric patients with ALL allografted after TBI-based conditioning regimen had a lower probability of relapse (20% vs 54%, $p = 0.004$) if FDC was achieved by day 30. Some studies considered that normal host hematopoietic cells in patients with MC might reduce the graft-versus-leukemia effect,[12,26] therefore exposing patients to a greater risk of relapse. In attempting to augment the graft-versus-leukemia effect, chimerism-guided DLI had been adopted as a pre-emptive treatment and might effectively prolong RFS.[7,27]

While the patients in our study had higher TRM (31%) compared to 8%–24% in historical reviews,[2,7–9,27] our TRM did not differ significantly depending on chimerism status on days 30 in contrary to other research.[2,9] We also observed that the patients in the early FDC group were prone to more

Table 2
Univariate analysis of risk factors for relapse.

Factors	Patients (n)	Relapse		Univariate	
		n	%	HR (95% CI)	*p*
Age					0.437
<16	19	7	37	1.00 (reference)	
≥16	36	11	30	0.685 (0.264–1.777)	
Diagnosis					0.908
B-ALL	26	9	35	1.00 (reference)	
Others	29	9	31	0.947 (0.375–2.389)	
Disease status before HSCT					0.026
Non-CR1	17	8	47	1.00 (reference)	
CR1	38	10	26	0.342 (0.132–0.879)	
Conditioning regimen					0.182
TBI-based (12Gy)	46	14	30	1.00 (reference)	
Chemotherapy-based	9	4	44	2.145 (0.699–6.580)	
Grading of aGVHD					0.978
Gr. 0–1	39	14	36	1.00 (reference)	
Gr. 2–4	16	4	25	1.016 (0.333–3.104)	
cGVHD					0.158
Nil to limited	39	16	41	1.00 (reference)	
Extensive	16	2	12	0.347 (0.080–1.509)	
Time to FDC					0.011
>30 days	24	12	50	1.00 (reference)	
≤30 days	31	6	19	0.273 (0.101–0.738)	

95% CI, 95% confidence interval; HR = hazard ratio; aGVHD = acute graft-versus-host disease; cGVHD = chronic graft-versus-host disease.

Table 3
Multivariate analysis of risk factors for relapse.

Factors	Patients (n)	Relapse		Multivariate	
		n	%	HR (95% CI)	*p*
Disease status before HSCT					0.024
Non-CR1	17	8	47	1.00 (reference)	
CR1	38	10	26	0.327 (0.124–0.865)	
Time to FDC					0.010
>30 days	24	12	50	1.00 (reference)	
≤30 days	31	6	19	0.264 (0.096–0.725)	

imminent deaths than those in the late group once relapses occurred, contributing to a shorter post-relapse survival, and might show an OS without significant difference. This phenomenon could be multi-factorial: faster achievement of FDC somewhat reflects greater immune-suppression imposed by heavier anti-leukemic therapy, thereby selecting ALL cell clones that are more aggressive in nature, and resulted in insufficient time to prompt subsequent treatment as overt relapses developed. We also find that patients with late FDC received more rescue DLI than those with early FDC, which may be an alternative explanation.

There are several limitations in our study, including the retrospective nature as the major limitation that would inevitably introduce bias in analyzing the survival outcome. The small patient number might prevent traditional risk factor for relapse in ALL, such as age, from reaching significance in the analysis. Both the graft CD34 cell number[2] and the degree of mismatching[7] of the donor-recipient information, having potential influence on chimerism kinetics, were incomplete or missing and could confound the predictive role of early FDC in survival analysis. In addition, we lacked the dosage and duration of immunosuppressive agents in treating acute GVHD, the frequency of DLI dosing, and characterization of chronic GVHD of each patient. The strengths of this study, namely single center cohort with a majority of adult ALL patients treated over an extended period with uniform TBI-based MAC followed by unmanipulated graft and CsA/MTX-based GVHD prophylactic protocol, empower our conclusion that FDC by days 30 after allo-HSCT act as a predictor for longer RFS in ALL patients. The results underscored the need to improve outcomes in ALL patients with late FDC. Based on our result, physicians might consider rapidly tapering immunosuppressive agents and even administering preemptive DLI in cases of persistent mixed chimera beyond days 30 after allo-HSCT. In the future, possibly in a larger cohort, researchers could prospectively investigate whether speeding up complete chimera by days 30 had beneficial impacts on survival outcomes.

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