Predictive Value of Dynamic Peri-Transplantation MRD Assessed By MFC Either Alone or in Combination with Other Variables for Outcomes of Patients with T-Cell Acute Lymphoblastic Leukemia*

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Summary: We performed a retrospective analysis to investigate dynamic peri-hematopoietic stem cell transplantation (HSCT) minimal/measurable residual disease (MRD) on outcomes in patients with T-cell acute lymphoblastic leukemia (T-ALL). A total of 271 patients were enrolled and classified into three groups: unchanged negative MRD pre- and post-HSCT group (group A), post-MRD non-increase group (group B), and post-MRD increase group (group C). The patients in group B and group C experienced a higher cumulative incidence of relapse (CIR) (42% vs. 71% vs. 16%, $P<0.001$) and lower leukemia-free survival (LFS) (46% vs. 21% vs. 70%, $P<0.001$) and overall survival (OS) (50% vs. 28% vs. 72%, $P<0.001$) than in group A, but there was no significant difference in non-relapse mortality (NRM) among three groups (14% vs. 12% vs. 8%, $P=0.752$). Multivariate analysis showed that dynamic peri-HSCT MRD was associated with CIR (HR=2.392, 95% CI, 1.816–3.151, $P<0.001$), LFS (HR=1.964, 95% CI, 1.546–2.496, $P<0.001$) and OS (HR=1.731, 95% CI, 1.348–2.222, $P<0.001$). We also established a risk scoring system based on dynamic peri-HSCT MRD combined with remission status pre-HSCT and onset of chronic graft-versus-host disease (GVHD). This risk scoring system could better distinguish CIR ($c=0.730$) than that for pre-HSCT MRD ($c=0.562$), post-HSCT MRD ($c=0.616$) and pre- and post-MRD dynamics ($c=0.648$). Our results confirm the outcome predictive value of dynamic peri-HSCT MRD either alone or in combination with other variables for patients with T-ALL.

Key words: peri-transplantation minimal residual disease; risk stratification; risk scoring system; T-cell acute lymphoblastic leukemia

Disease recurrence remains one of the most common causes of death in patients with acute lymphoblastic leukemia (ALL)^[1–6], especially for those with T-cell ALL (T-ALL)^[7], who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT). Presently, a number of variables^[2, 7–15], such as remission status pre-HSCT, immunophenotype of ALL, minimal/measurable residual disease (MRD)

and chronic graft-versus-host disease (GVHD), have been reported to be associated with relapse either in chemotherapy settings or in allo-HSCT modalities. Among these, MRD can be used not only for relapse prediction^[2, 15, 16] but also for treatment selection^[3, 15, 17]. Unfortunately, due to 10%–15% of total ALL cases were T-ALL^[18] most studies about the correlation of MRD with clinical outcomes focused on total ALL patients or those with B-cell ALL^[2, 3, 11, 12, 15, 16, 19–21]. For patients with T-ALL, the effects of positive MRD at day 15 and 29 after therapy^[7, 22], post-induction^[6, 23] as well as pre-HSCT^[2, 10, 24] on relapse and survival have been previously demonstrated. However, there are limited data on the effects of peri-HSCT MRD on outcomes for ALL patients^[12, 20, 25] particularly for T-ALL patients. Therefore, we performed a retrospective study to investigate the effects of dynamic peri-HSCT MRD on

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transplantation outcomes in patients with T-ALL. In addition, we also tried to establish a risk score principally based on the dynamic peri-HSCT MRD combined with other parameters, such as remission status pre-HSCT and onset of chronic GVHD demonstrated by others^[12, 26, 27] and us^[2, 16, 19], which might provide better relapse risk determination for T-ALL patients.

1 PATIENTS AND METHODS

1.1 Study Design

This retrospective study included T-ALL subjects who were enrolled at the Peking University People's Hospital between January 2010 and December 2018. For patients with human leukocyte antigen (HLA)-matched sibling donors (MSDs), MSDs were chosen. If cases without MSDs, HLA-matched unrelated donors (MUDs) were chosen. If cases without MSDs and MUDs, then haploidentical donors were chosen^[28, 29]. All of the included subjects signed an informed consent form. The study protocol was in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Peking University. All of the cases were treated according to the transplant protocol as previously described^[1, 17, 30].

1.2 Transplant Procedures

Recombinant human granulocyte colony-stimulating factor (G-CSF; 5 µg/kg per day for 5 days) were administered to healthy donors for bone marrow stem cells (BMSCs, collected on day 4 after G-CSF) and peripheral blood stem cells (PBSCs, collected on day 5 after G-CSF) mobilization^[21, 30]. Subjects received BMSCs and/or PBSCs as allografts.

All patients were treated with a myeloablative conditioning regimen^[1, 17, 21, 30]. For patients with haploidentical donors (HIDs), the conditioning regimen was given as follows: cytarabine (4 g/m² per day) intravenously on days -10 to -9; busulfan (3.2 mg/kg per day) intravenously on days -8 to -6; cyclophosphamide (1.8 g/m² per day), intravenously on days -5 to -4; Me-CCNU (250 mg/m² per day), orally once on day -3; and ATG (thymoglobulin, 2.5 mg/kg per day, Sang Stat, France) intravenously on days -5 to -2. Patients with MSDs received hydroxycarbamide (80 mg/kg) orally on day -10 and a lower dose of cytarabine (2 g/m² per day) on day -9, but otherwise, an identical regimen to the HID patients without ATG was employed.

1.3 MFC Detection of MRD

Bone marrow aspirate samples were obtained as part of the baseline assessment before SCT, as well as 1, 2, 3, 4.5, 6, 9, and 12 months posttransplantation and at 6-month intervals thereafter according to previous studies^[2, 16, 21, 30]. Six- to eight-color MFC was performed for MRD evaluation according to previous studies^[2, 16, 21]. A panel of antibody combinations recognizing cTdT,

mCD3, cCD3, CD5, CD7, CD34, CD45, and CD2 or CD99 was used for MRD determination. Any measurable level of MRD was considered positive, otherwise was defined as negative. The definition for quantitative dynamics of pre-MRD and post-MRD included: (1) post-MRD increase after allograft compared with the pre-HSCT baseline; (2) post-MRD non-increase was defined as not meeting the criteria of (1) and (3); (3) unchanged negative MRD pre- and post-HSCT.

1.4 Methods for MRD Intervention and Relapse Treatment

Donor lymphocyte infusion (DLI) was performed as described previously by our group^[1, 17, 31]. Other methods for positive MRD intervention and relapse treatment, such as interferon-γ (IFN-γ), were administered according to our previous studies^[1, 17, 31].

1.5 Outcomes

The primary study end point was the cumulative incidence of leukemia relapse. The secondary end points were the cumulative incidence of non-relapse mortality (NRM) and the probabilities of leukemia-free survival (LFS) and overall survival (OS).

The engraftment, infection, NRM, relapse, LFS, and OS were defined according to our previous studies^[1, 17, 31, 32]. The definition and grades of acute GVHD were based on the pattern and severity of organ involvement^[32]. The chronic GVHD was defined and graded according to the National Institute of Health criteria^[33].

1.6 Statistical Analysis

Patient characteristics were compared between the negative MRD and positive MRD groups with the χ^2 statistic for categorical variables and the Mann-Whitney test for continuous variables. Cumulative incidence curves were used in a competing risk setting, with relapse treated as a competing event to calculate NRM probabilities, and with death from any cause as a competing risk for GVHD, engraftment, and relapse. The probabilities of LFS and OS were estimated with the Kaplan-Meier method. The variables in table 1 were included in the univariate analysis. Only variables with $P < 0.1$ were included in a Cox proportional hazards model with time-dependent variables. We calculated C-statistics (c), whereby a c value of 1.0 indicates perfect discrimination, and a c value of 0.5 is equivalent to chance^[34]. Unless otherwise specified, P values were based on two-sided hypothesis tests. Alpha was set at 0.05. Most analyses were performed with SPSS software, version 16.0 (Mathsoft, USA).

2 RESULTS

2.1 Patient Characteristics and Transplant Outcomes

A total of 271 consecutive cases were included in this study. The characteristics of all of the cases,

Table 1 Characteristics of patients and donors

Characteristics	All patients <i>n</i>	Pre-MRDneg	Pre-MRDpos	<i>P</i> value	Post-MRDneg	Post-MRDpos	<i>P</i> value
Median age (range), years	271	227	44		236	35	
Male sex, <i>n</i> (%)	23 (2–58) 202 (74.5%)	23 (2–58) 170 (74.9%)	21.5 (14–46) 32 (72.7%)	0.416 0.763	23 (2–58) 172 (72.9%)	19 (3–41) 30 (85.7%)	0.167 0.104
Diagnosis, <i>n</i> (%)				NS			NS
T-ALL	271 (100%)	227 (100%)	44 (100%)		236 (100%)	35 (100%)	
Disease status, <i>n</i> (%)				0.143			0.328
CR1	249 (91.9%)	211 (93.0%)	38 (86.4%)		215 (91.1%)	34 (97.1%)	
≥CR2	22 (8.1%)	16 (7.0%)	6 (13.6%)		21 (8.9%)	1 (2.9%)	
Median time from diagnosis to transplant (range), months	6.5 (3–49)	6.5 (3–49)	6.5 (4–47)	0.299	6.5 (3–49)	6.5 (3.5–14)	0.378
Conditioning regimen, <i>n</i> (%)				NS			NS
MA	271 (100%)	227 (100%)	44 (100%)		236 (100%)	35 (100%)	
Transplant modalities				0.014			0.462
MSDT	51 (18.8%)	48 (21.1%)	3 (6.8%)		46 (19.5%)	5 (14.3%)	
Haplo-SCT	220 (81.2%)	179 (78.9%)	41 (93.2%)		190 (80.5%)	30 (85.7%)	
The median (range) level of pre-MRD	0 (0–4.98%)	0	0.26% (0.0019%–4.98%)		0	0	
The median (range) level of pos-MRD	0 (0–7.76%)	0	0		0	0.17% (0.003%–7.76%)	
HLA-A, B, DR mismatched grafts, <i>n</i> (%)				0.029			0.928
0	52 (19.2%)	48 (21.1%)	4 (9.1%)		46 (19.5%)	6 (17.1%)	
1	12 (4.5%)	12 (5.3%)	0		11 (4.7%)	1 (2.9%)	
2	34 (12.5%)	28 (12.3%)	6 (13.6%)		29 (12.3%)	5 (14.3%)	
3	173 (63.8%)	139 (61.3%)	34 (77.3%)		150 (63.6%)	23 (65.7%)	
Donor-recipient sex matched grafts, <i>n</i> (%)				0.019			0.024
Male-male	138 (50.9%)	111 (48.9%)	27 (61.4%)		112 (47.5%)	26 (74.3%)	
Male-female	46 (17.0%)	35 (15.4%)	11 (25.0%)		43 (18.2%)	3 (8.6%)	
Female-male	66 (24.4%)	61 (26.9%)	5 (11.4%)		62 (26.3%)	4 (11.4%)	
Female-female	21 (7.7%)	20 (8.8%)	1 (2.3%)		19 (8.1%)	2 (5.7%)	
Donor-recipient relationship, <i>n</i> (%)				0.085			0.138
Parent-child	157 (57.9%)	127 (56.0%)	30 (68.2%)		134 (56.7%)	23 (65.7%)	
Sibling-sibling	102 (37.6%)	91 (40.1%)	11 (25.0%)		92 (39.0%)	10 (28.6%)	
Child-parent	8 (3.0%)	6 (2.6%)	2 (4.5%)		7 (3.0%)	1 (2.9%)	
Others	4 (1.5%)	3 (1.3%)	1 (2.3%)		3 (1.3%)	1 (2.9%)	

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Characteristics	All patients	Pre-MRDneg	Pre-MRDpos	P value	Post-MRDneg	Post-MRDpos	P value
ABO matched grafts, <i>n</i> (%)				0.050			0.085
Matched	154 (56.8%)	122 (53.7%)	32 (72.7%)		130 (55.1%)	24 (68.6%)	
Major mismatch	52 (19.2%)	49 (27.6%)	3 (6.8%)		49 (20.8%)	3 (8.6%)	
Minor mismatch	49 (18.1%)	42 (18.5%)	7 (15.9%)		45 (19.1%)	4 (11.4%)	
Bi-directional mismatch	16 (5.9%)	14 (6.2%)	2 (4.5%)		12 (5.1%)	4 (11.4%)	
Cell compositions in allografts							
Infused nuclear cells, (range) 10 ⁶ /kg	8.35 (3.29–17.6)	8.32 (3.29–17.60)	8.48 (5.83–13.97)	0.526	8.37 (3.29–17.60)	8.29 (5.80–11.51)	0.835
Infused CD34 ⁺ cells, (range) 10 ⁶ /kg	2.44 (0.50–12.44)	2.54 (0.50–12.44)	2.11 (0.88–4.98)	0.058	2.43 (0.50–12.44)	2.62 (1.54–6.56)	0.133
DLI and IFN- γ for MRD intervention after transplant, <i>n</i> (%)	30 (11.1%)	21 (9.3%)	9 (22.7%)	0.030	0	5 (14.3%)	<0.001

Haplo-SCT: haploidentical stem cell transplantation; pre-MRD: pre-transplantation minimal residual disease; neg: negative; pos: positive; NS: no significance; ALL: acute lymphoblastic leukemia; CR: complete remission; MA: myeloablative conditioning regimen; HLA: human leukocyte antigen; DLI: donor lymphocyte infusions

including positive and negative pre-HSCT or post-HSCT MRD groups, are summarized in table 1. There was higher percentage of cases in the positive pre-HSCT MRD group receiving haploidentical HSCT than in the negative pre-HSCT group ($P=0.014$). However, our previous studies demonstrated that, for ALL patients or acute myeloid leukemia patients, haploidentical HSCT had a superior anti-leukemia activity to MSDT^[15, 17, 19, 35]. Higher percentages of patients in the positive MRD groups received IFN- γ or DLI for relapse intervention than those of the negative MRD groups either pre- or post-HSCT ($P=0.030$ or <0.001 , respectively) (table 1).

All patients had sustained, full donor chimerism. The cumulative 100-day incidence of platelet engraftment and acute GVHD grades II to IV was 93% (95% CI, 90%–96%) and 22% (95% CI, 17%–27%), respectively. After a median follow-up of 32 months (range, 0.93–127 months) and 52 months (range, 19–127 months), for all cases and live cases, respectively, the 3-year cumulative incidence of chronic GVHD was 49% (95% CI, 42%–56%). The 3-year NRM and cumulative incidence of relapse (CIR) were 13% (95% CI, 9%–17%) and 26% (95% CI, 21%–31%), respectively. The 3-year probabilities of LFS and OS were 62% (95% CI, 56%–68%) and 64% (95% CI, 58%–70%), respectively.

2.2 Effects of Peri-HSCT MRDs on Outcomes

We first investigated the effects of pre-HSCT MRDs or post-pre-HSCT MRDs on transplant outcomes in total cases. The patients in the positive pre-HSCT MRD group experienced higher CIR (45% vs. 22%, $P=0.003$), lower LFS (44% vs. 65%, $P=0.012$) and OS (50% vs. 67%, $P=0.051$) than those in the negative pre-HSCT MRD group, but there was no significant difference in NRM between the positive and negative pre-HSCT MRD groups (11% vs. 13%, $P=0.805$). Patients with positive post-HSCT MRDs had higher CIR (72% vs. 19%, $P<0.001$), lower LFS (22% vs. 67%, $P<0.001$) and OS (32% vs. 69%, $P<0.001$) than those with negative post-HSCT MRDs, but no significant difference was found in NRM (6% vs. 14%, $P=0.193$) (fig. 1 and table 2). Multivariate analysis showed that both positive pre-HSCT MRD and positive post-HSCT MRD were independently associated with leukemia relapse (HR=1.932, 95% CI, 1.117–3.342, $P=0.019$ and HR=4.547, 95% CI, 2.717–7.608, $P<0.001$, respectively) and LFS (HR=1.932, 95% CI, 1.117–3.342, $P=0.019$ and HR=2.920, 95% CI, 1.848–4.613, $P<0.001$, respectively), but only positive post-HSCT MRD was correlated with OS (HR=2.448, 95% CI, 1.525–3.931, $P<0.001$). In addition, onset of chronic GVHD was associated with leukemia relapse (HR=0.399, 95% CI, 0.238–0.670, $P=0.001$) and LFS (HR=0.357, 95% CI, 0.231–0.553, $P<0.001$).

We further evaluated the effects of peri-HSCT

Fig. 1 Outcomes of T-ALL patients who underwent allogeneic stem cell transplantation according to different prognostic variables. Cumulative incidence of 3-year relapses according to pre-HSCT MRD (A), post-HSCT MRD (C), dynamic peri-HSCT MRD (E), and risk scores (G) principally based on dynamic peri-HSCT MRD. Probabilities of 3-year leukemia-free survival according to pre-HSCT MRD (B), post-HSCT MRD (D), dynamic peri-HSCT MRD (F), and risk scores (H) principally based on dynamic peri-HSCT MRD. T-ALL: T cell acute lymphoblastic leukemia; HSCT: hematopoietic stem cell transplantation; MRD: minimal/measurable residual disease

Table 2 Transplant outcomes for ALL patients who underwent allo-SCT in subgroup cases of total patients (n=271)

	II – IV acute aGVHD	Chronic GVHD	CIR at 3 years	NRM at 3 years	LFS at 3 years	OS at 3 years
Total patients						
Pre-MRDpos (n=44)	20% (8%–32%)	61% (43%–79%)	45% (29%–60%)*	11% (2%–20%)	44% (29%–59%)**	50% (34%–65%)#
Pre-MRDneg (n=227)	22% (16%–28%)	47% (40%–54%)	22% (17%–27%)	13% (9%–17%)	65% (59%–71%)	67% (60%–73%)
Total patients						
Post-MRDpos (n=35)	23% (9%–37%)	56% (34%–78%)	72% (56%–87%)###	6% (0–15%)	22% (8%–36%)###	32% (16%–48%)###
Post-MRDneg (n=236)	21% (15%–27%)	49% (42%–56%)	19% (14%–24%)	14% (9%–18%)	67% (61%–74%)	69% (63%–75%)
Total patients						
Post-MRD increase (n=27)	22% (6%–38%)	65% (33%–97%)	71% (53%–89%)	8% (0–19%)	21% (5%–37%)	28% (10%–45%)
Post-MRD non-increase (n=42)	21% (9%–33%)	59% (41%–77%)	42% (26%–58%)	12% (2%–23%)	46% (31%–62%)	50% (34%–66%)
Unchanged negative MRD pre- and post-HSCT (n=202)	22% (16%–28%)	47% (39%–55%)	16% (11%–22%)	14% (9%–18%)	70% (64%–76%)	72% (66%–78%)
Total patients						
Risk score						
Low risk (n=82)	28% (18%–38%)	100% ^s	7% (2%–13%)	6% (1%–12%)	86% (79%–94%)	88% (80%–95%)
Intermediate risk (n=126)	19% (12%–26%)	20% (12%–28%)	23% (15%–30%)	15% (9%–21%)	62% (54%–71%)	65% (56%–74%)
High risk (n=42)	14% (3%–25%)	39% (19%–59%)	51% (35%–67%)	14% (4%–25%)	35% (20%–49%)	40% (24%–56%)
Very high risk (n=21)	24% (5%–43%)	8% (6%–10%)	67% (45%–89%)	21% (1%–41%)	12% (0–27%)	12% (0–27%)

*P=0.003 vs. pre-MRDneg group. **P=0.012 vs. pre-MRDneg group. #P=0.051 vs. pre-MRDneg group. ###P<0.001 vs. post-MRDneg group. ^sP<0.001 vs. other groups.

ALL: acute lymphoblastic leukemia; allo-SCT: allogeneic stem cell transplantation; CIR: cumulative incidence of relapse; NRM: non-relapse mortality; LFS: leukemia-free survival; OS: overall survival; MRD: minimal residual disease; Pre-MRDpos: positive pre-HSCT MRD; Pre-MRDneg: negative pre-HSCT MRD; Post-MRDpos: positive post-HSCT MRD; Post-MRDneg: negative post-HSCT MRD

MRD on transplant outcomes. The total cases were classified as unchanged negative MRD pre- and post-HSCT group (group A), post-MRD non-increase group (group B), and post-MRD increase group (group C), respectively. The CIR in group B and group C was significantly higher (42% vs. 71% vs. 16%, $P<0.001$), LFS was significantly lower (46% vs. 21% vs. 70%, $P<0.001$) and OS lower (50% vs. 28% vs. 72%, $P<0.001$), but NRM was comparable among three groups (14% vs. 12% vs. 8%, $P=0.752$) (fig. 1 and table 2). Multivariate analysis showed that peri-HSCT MRD was associated with CIR (HR=2.392, 95% CI, 1.816–3.151, $P<0.001$), LFS (HR=1.964, 95% CI, 1.546–2.496, $P<0.001$) and OS (HR=1.731, 95% CI, 1.348–2.222, $P<0.001$) (table 3). In addition, onset of chronic GVHD was associated with leukemia relapse (HR=0.377, 95% CI, 0.223–0.637, $P<0.001$) and LFS (HR=0.344, 95% CI, 0.222–0.535, $P<0.001$). The predictive value of peri-HSCT MRD on outcomes was also observed after analysis in the adult and pediatric subgroups (data not shown).

2.3 A Risk Score for CIR Prediction

We applied multivariate Cox regression analysis with stepwise forward selection based on the data of total patients. The final model included remission status before transplantation (CR1 scores: 0; $\geq$ CR2 scores: 1), onset of chronic GVHD (with chronic GVHD scores: 0; without chronic GVHD scores: 1) and dynamics of pre- and post-HSCT MRD (pre-MRDneg and post-MRDneg, MRD non-increase, and MRD increase, scores: 0, 1, and 2, respectively). According to the risk score categories, we classified each patient into one of four prognostic risk groups: low-risk (score=0), intermediate-risk (score=1), high-risk (score=2) and very high-risk (score=3).

The 3-year CIR (7%, 23%, 51%, and 67%, respectively, $P<0.001$), NRM (6%, 15%, 14%, and 21%, respectively, $P<0.001$), LFS (86%, 62%, 35%, and 12%, respectively, $P<0.001$) and OS (88%, 65%, 40%, and 12%, respectively, $P<0.001$) in the low-risk, intermediate-risk, high-risk and very high-risk groups were listed in table 2 and fig. 1. Multivariate analysis

Table 3 Univariate and multivariate analysis of factors associated with outcomes of ALL patients who underwent allo-SCT considering dynamic peri-transplantation MRD ($n=271$)

Covariate	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>P</i> value	HR	95% CI	<i>P</i> value
Relapse						
Disease status (CR $\geq$ 2 vs. CR1)	2.929	1.450–5.918	0.003	3.253	1.596–6.628	0.001
Chronic GVHD (yes vs. no)	0.434	0.260–0.726	0.001	0.375	0.223–0.631	<0.001
Recipient age	0.976	0.953–1.000	0.047			
Dynamic peri-SCT MRD						
MRD increase	5.384	3.051–9.501	<0.001	6.076	3.420–10.792	<0.001
MRD non-increase	2.923	1.627–5.249	<0.001	3.353	1.875–6.067	<0.001
Pre-MRDneg and post-MRDneg		1			1	
Non-relapse mortality						
Disease status (CR $\geq$ 2 vs. CR1)	3.884	1.683–8.963	0.001	4.621	1.960–10.892	<0.001
Platelet engraftment	2.875	1.244–6.562	0.013	2.905	1.263–6.681	0.012
Neutrophil engraftment	1.809	0.895–3.656	0.099			
Recipient age	1.028	0.997–1.059	0.074	2.068	1.026–4.170	0.042
Leukemia-free survival						
Disease status (CR $\geq$ 2 vs. CR1)	3.494	2.044–5.972	<0.001	3.598	2.093–6.185	<0.001
Gender (female vs. male)	0.565	0.340–0.940	0.028			
Chronic GVHD (yes vs. no)	0.375	0.243–0.579	<0.001	0.344	0.222–0.535	<0.001
Dynamic peri-SCT MRD						
MRD increase	3.202	1.944–5.275	<0.001	3.685	2.225–6.104	<0.001
MRD non-increase	2.057	1.261–3.535	0.004	2.358	1.439–3.863	0.001
Pre-MRDneg and post-MRDneg		1			1	
Overall survival						
Disease status (CR $\geq$ 2 vs. CR1)	3.585	2.088–6.157	<0.001	3.676	2.137–6.322	<0.001
Gender (female vs. male)	0.589	0.348–0.995	0.048			
Dynamic peri-SCT MRD						
MRD increase	2.803	1.663–4.723	<0.001	2.922	1.732–4.929	<0.001
MRD non-increase	1.943	1.165–3.238	0.011	1.891	1.133–3.145	0.015
Pre-MRDneg and post-MRDneg		1			1	

*All variables were first included in the univariate analysis; only variables with $P<0.1$ were included in the Cox proportional hazards model with time-dependent variables.

ALL: acute lymphoblastic leukemia; Allo-SCT: allogeneic stem cell transplantation; MRD: minimal/measurable residual disease; HR: hazard ratio; CI: confidence interval; CR: complete remission; GVHD: graft-versus-host disease; peri-SCT: peri-stem cell transplantation; pre-MRDneg: negative pre-transplantation MRD; post-MRDneg: negative post-transplantation MRD

showed that the risk score was associated with CIR (HR=2.574, 95% CI, 2.013–3.291, $P<0.001$), NRM (HR=1.734, 95% CI, 1.198–2.508, $P=0.004$), LFS (HR=2.229, 95% CI, 1.822–2.728, $P<0.001$) and OS (HR=2.164, 95% CI, 1.755–2.667, $P<0.001$) (table 4). The risk score system could better distinguish CIR ($c=0.730$) than that for pre-HSCT MRD ($c=0.562$), post-HSCT MRD ($c=0.616$) and pre- and post-MRD dynamics ($c=0.648$). The value of the risk score for CIR prediction was also observed after analysis in the adult and pediatric subgroups (data not shown).

3 DISCUSSION

In consistent with previous studies either in total ALL patients^[16,20] or B-ALL subgroup patients^[36], we, in the present study, found that dynamic peri-HSCT MRD in patients with T-ALL could be better for discrimination of relapse risk than that of pre-HSCT MRD or post-HSCT MRD. In addition, we showed that a risk score

principally based on dynamic peri-transplantation MRD could further achieve better relapse stratification than dynamic peri-HSCT MRD alone ($c\text{-index}=0.730$ vs. 0.648). Overall, our results add new evidence for the application of MRD, suggesting the usefulness of dynamic peri-HSCT MRD for stratification of T-ALL patients with high risk recurrence.

In a recent study including 477 B-ALL patients who underwent allo-HSCT, Cao *et al*^[19] demonstrated that post-HSCT MRD, but not pre-HSCT MRD was associated with higher CIR and shorter survival after multivariate analysis. In contrast to the result by Cao *et al*^[19], we found that both pre- and post-MRD could be used for discriminating patients into different relapse risk groups for T-ALL patients, although post-HSCT MRD was better than pre-HSCT MRD in predicting leukemia relapse (table 2 and fig. 1). The above-mentioned differences might be related to the higher CIR and shorter survival in patients with T-ALL who underwent allografts than in B-ALL patients who

Table 4 Univariate and multivariate analysis of factors associated with outcomes of ALL patients who underwent allo-SCT considering a risk score based on dynamic peri-transplantation MRD ($n=271$)

Covariate	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>P</i> value	HR	95% CI	<i>P</i> value
Relapse						
Recipient age	0.976	0.953–1.000	0.047			
Risk score						
Low risk		1			1	
Intermediate risk	3.670	1.519–8.865	0.004	3.670	1.519–8.865	0.004
High risk	10.309	4.154–25.583	<0.001	10.309	4.154–25.583	<0.001
Very high risk	19.412	7.417–50.803	<0.001	19.412	7.417–50.803	<0.001
Non-relapse mortality						
Platelet engraftment	2.875	1.244–6.562	0.013	3.331	1.438–7.717	0.005
Neutrophil engraftment	1.809	0.895–3.656	0.099			
Recipient age	1.028	0.997–1.059	0.074	1.036	1.004–1.069	0.027
Risk score						
Low risk		1			1	
Intermediate risk	2.792	1.042–7.479	0.041	3.285	1.217–8.870	0.019
High risk	2.802	0.854–9.193	0.089	3.282	0.990–10.881	0.052
Very high risk	4.381	1.172–16.384	0.028	7.463	1.950–28.349	0.003
Leukemia-free survival						
Gender (female vs. male)	0.565	0.340–0.940	0.028			
Risk score						
Low risk		1			1	
Intermediate risk	3.333	1.728–6.246	<0.001	3.333	1.728–6.246	<0.001
High risk	7.058	3.496–14.294	<0.001	7.058	3.496–14.294	<0.001
Very high risk	12.928	6.077–27.503	<0.001	12.928	6.077–27.503	<0.001
Overall survival						
Gender (female vs. male)	0.589	0.348–0.995	0.048			
Risk score						
Low risk		1			1	
Intermediate risk	3.293	1.655–6.554	0.001	3.293	1.655–6.554	0.001
High risk	6.290	3.004–13.167	<0.001	6.290	3.004–13.167	<0.001
Very high risk	12.364	5.677–26.927	<0.001	12.364	5.677–26.927	<0.001

*All variables were first included in the univariate analysis; only variables with $P<0.1$ were included in the Cox proportional hazards model with time-dependent variables.

ALL: acute lymphoblastic leukemia; Allo-SCT: allogeneic stem cell transplantation; MRD: minimal/measurable residual disease; HR: hazard ratio; CI: confidence interval

received allo-HSCT^[2]. However, the results of the present study are consistent with those of previous study including both T-ALL and B-ALL cases receiving allograft, reported by Bader *et al*^[12]. They reported a higher c-index for the post-HSCT MRD than that of pre-HSCT MRD (c-index=0.649 vs. 0.612).

Considering MRD was detected at different time points, several studies focused on the dynamic MRD change in predicting CIR for patients with acute leukemia^[12, 16, 20, 25, 37, 38]. For 279 patients with AML receiving allografts, pre-HSCT MRD and post-HSCT MRD were determined by MFC. Zhou *et al*^[38] found that patients with increased MRD levels around the time of transplantation experienced higher CIR and shorter survival than those with decreased MRD levels or those with negative pre-HSCT MRD and negative post-HSCT MRD. We have also confirmed the results reported by Zhou *et al*^[38] either in total AML patients or in pediatric and young adult ALL cases. Here, we confirmed the superiority of dynamic peri-HSCT MRD compared to pre-HSCT MRD and post-HSCT MRD in T-ALL patients receiving allo-HSCT (table 2 and fig. 1). The results of our previous study^[16] and other studies^[20, 38] suggest that dynamic peri-HSCT MRD should be routinely used for CIR discrimination in allograft settings for patients with acute leukemia.

In a large cohort of study including 616 pediatric and young adult ALL patients receiving allografts, Bader *et al*^[12] showed that these patients could be classified into three different relapse risk groups according to a prognostic risk score established based on remission status before transplantation, conditioning regimen and pre-HSCT MRD. In the present study, except for dynamic MRD peri-transplantation, remission status pre-HSCT and onset of chronic GVHD were also independently correlated with CIR and survival. We found, in subgroup patients with T-ALL who underwent allograft, a risk score principally based on dynamic peri-HSCT MRD as well as remission status and chronic GVHD could further classify patients into four subgroups with different CIR and survival (table 2 and fig. 1). Therefore, the results in the present study and previous studies shown by others^[12, 39] and us^[36] suggest that risk scores based on MRD and other variables could be better in predicting transplant outcomes for ALL patients, especially those of T-ALL with a CIR more than 50% (table 2 and fig. 1).

There are limitations of our study. First, this is a retrospective, single center study. Second, the present study only enrolled T-ALL patients who underwent haploidentical HSCT and MSDT. Third, we did not perform subgroup analysis of cases who received MSDT due to the small number of patients in subgroup. Therefore, a prospective, multicenter study with training and validation sets is needed to further confirm whether our findings are suitable for T-ALL cases

who either received haploidentical HSCT (including haploidentical HSCT based on immune tolerance induced by post-transplantation cyclophosphamide), MSDT, MUD transplantation^[5] or umbilical cord blood transplantation^[40].

In summary, our results suggest a superiority of dynamic peri-HSCT MRD to single time point, including pre-HSCT MRD and post-HSCT MRD, in relapse risk stratification for patients with T-ALL. We further suggest that T-ALL patients who would experience the worst outcome could be discriminated by a risk score principally based on dynamic peri-HSCT MRD, for these cases, novel strategies are needed to improve the transplant outcomes.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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