A risk score system for stratifying the risk of relapse in B cell acute lymphocytic leukemia patients after allogeneic stem cell transplantation

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Abstract
Background: For patients with B cell acute lymphocytic leukemia (B-ALL) who underwent allogeneic stem cell transplantation (allo-SCT), many variables have been demonstrated to be associated with leukemia relapse. In this study, we attempted to establish a risk score system to predict transplant outcomes more precisely in patients with B-ALL after allo-SCT.

Methods: A total of 477 patients with B-ALL who underwent allo-SCT at Peking University People’s Hospital from December 2010 to December 2015 were enrolled in this retrospective study. We aimed to evaluate the factors associated with transplant outcomes after allo-SCT, and establish a risk score to identify patients with different probabilities of relapse. The univariate and multivariate analyses were performed with the Cox proportional hazards model with time-dependent variables.

Results: All patients achieved neutrophil engraftment, and 95.4% of patients achieved platelet engraftment. The 5-year cumulative incidence of relapse (CIR), overall survival (OS), leukemia-free survival (LFS), and non-relapse mortality were 20.7%, 70.4%, 65.6%, and 13.9%, respectively. Multivariate analysis showed that patients with positive post-transplantation minimal residual disease (MRD), transplanted beyond the first complete remission (≥CR2), and without chronic graft-versus-host disease (cGVHD) had higher CIR (P < 0.001, P = 0.004, and P < 0.001, respectively) and worse LFS (P < 0.001, P = 0.017, and P < 0.001, respectively), and OS (P < 0.001, P = 0.009, and P < 0.001, respectively) than patients without MRD after transplantation, transplanted in CR1, and with cGVHD. A risk score for predicting relapse was formulated with the three above variables. The 5-year relapse rates were 6.3%, 16.6%, 55.9%, and 81.8% for patients with scores of 0, 1, 2, and 3 (P < 0.001), respectively, while the 5-year LFS and OS values decreased with increasing risk score.

Conclusion: This new risk score system might stratify patients with different risks of relapse, which could guide treatment.

Keywords: B cell acute lymphocytic leukemia; Allogeneic stem cell transplantation; Minimal residual disease; Disease status; chronic graft-versus host disease; Patient outcome

Introduction
Outcomes of acute lymphoblastic leukemia (ALL) have improved with the development of treatment measures.[1,2] However, relapse remains the major cause of treatment failure in patients with ALL who either exclusively received chemotherapy or additionally underwent allogeneic stem cell transplantation (allo-SCT).[3,4] For allo-SCT cases, almost all available studies have demonstrated that positive measurable/minimal residual disease (MRD) before[5-12] and after[13-25] transplantation was related to a higher cumulative incidence of relapse (CIR) in both adult and pediatric ALL. A previous study[18] also showed that the positive MRD at the time points, including days +30, +60, +90, +180, and +365 after allo-SCT, were inversely correlated with event-free survival and positively correlated with CIR in pediatric ALL. More recently, we reported the association of both quantitative and qualitative pre-transplantation MRD (pre-MRD)[11] as well as post-transplantation MRD (post-MRD)[25] with increased CIR and inferior survival in patients with ALL who underwent haploidentical allograft transplantation.

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For patients with ALL who underwent allo-SCT, other variables in addition to post-MRD have also been demonstrated to be associated with leukemia relapse. For example, patients in the first complete remission (CR1) have better outcomes than those in CR2 or those with advanced disease stage. Meanwhile, studies reported by others have shown that the onset of graft-versus-host disease (GVHD) after allo-SCT can reduce CIR. Recent studies found that acute graft-versus-host disease (aGVHD) or chronic graft-versus-host disease (cGVHD) could reduce CIR for patients with positive post-MRD as well as for patients transplanted with advanced disease (≥CR3 or active disease).

However, there are rare data regarding the prognostic significance of the combination of disease status with pre-MRD or post-MRD and GVHD in patients with ALL who are receiving allo-SCT. In addition, long-term survival has improved in patients with ALL who undergo current treatment regimens, but outcomes for T cell ALL are still worse than those for B cell acute lymphocytic leukemia (B-ALL). Therefore, we attempted to establish a risk score based on pre-MRD or post-MRD determined by multiparametric flow cytometry (MFC) and disease status as well as cGVHD to explore whether it could predict transplant outcomes more precisely in patients with B-ALL after allo-SCT.

Materials and Methods

Ethical approval

This study met the guidelines of the Helsinki Declaration of 1975 and was approved by the Ethics Committee of Peking University People’s Hospital (No. 2014PH0806-01). Informed consent was obtained from all patients or their guardians, and donors.

Patients and study design

This is a retrospective study. A total of 477 patients at the Peking University People’s Hospital from December 2010 to December 2015 were enrolled. Donor selection and transplant protocol were performed as previously described. Patients with B-ALL who underwent allo-SCT were included, and patients who lost follow-ups were excluded.

Transplant protocol

Granulocyte colony-stimulating factor (G-CSF; 5 μg per kilogram of body weight per day for 5 days) was used to mobilize granulocytes from the bone marrow (G-BM) and the peripheral blood (G-PB). The target mononuclear cell count was ≥6 × 10⁸ per kilogram of recipient weight. Unmanipulated BM (harvested on day 4 after G-CSF) and PB stem cells (PBSCs, harvested on day 5 after G-CSF) were infused into the recipient on the day of collection. All patients who underwent haploidentical blood and marrow transplantation (HBMT) or HLA-matched sibling donor transplantation (MSDT) received both G-BM and G-PB, while patients who underwent HLA-matched unrelated donor transplantation (MUDT) received G-PB.

The conditioning therapy for the HBMT group was as follows: cytarabine (4 g·m⁻²·d⁻¹) intravenously on days −10 to −9; busulfan (3.2 mg·kg⁻¹·d⁻¹) intravenously on days −8 to −6; cyclophosphamide (1.8 g·m⁻²·d⁻¹) intravenously on days −5 to −4; and methotrexate (5 mg·kg⁻¹·d⁻¹) orally on days −10 and a lower dose of cytarabine (2 g·m⁻²·d⁻¹) on day −9, but otherwise, an identical regimen to that of the HBMT patients without ATG was used. In the MUDT group, patients received the same regimen as the MSDT group but with the addition of ATG, as in the HBMT group. All patients received immunosuppressive agents, including cyclosporine A, mycophenolate mofetil, and short-term methotrexate, to prevent GVHD.

MFC detection of MRD

The first day after stem cell infusion was defined as day 1. Multicolor MFC was performed in all patients on bone marrow aspirate samples that were obtained as part of the baseline assessment at diagnosis, across the duration of chemotherapy, before and around days +30, +60, +90, +120, and +180 or more after transplantation. In this study, we focused on MRD status pre- and post-transplantation. Different antibody combinations were used as described previously. Residual disease ≥0.001% detected by MFC at any time point before and after allo-SCT was considered pre-MRD or post-MRD positive (MRD⁺), while persistent negative MRD at all time points after transplantation was defined as MRD negative (MRD⁻).

Prevention and treatment of relapse

Once MRD turned positive or hematological relapse occurred, some measures were taken to prevent or treat relapse, including immunosuppression tapering, targeted drugs (such as tyrosine kinase inhibitors), interferon, donor lymphocyte infusion (DLI) with previous chemotherapy, and chimeric antigen receptor T cell immunotherapy.

Definitions and assessments

The diagnostic criteria for ALL followed the World Health Organization 2008 criteria. The primary study endpoint was the cumulative incidence of leukemia relapse. The secondary endpoints were the cumulative incidences of non-relapse mortality (NRM), the probabilities of leukemia-free survival (LFS), and overall survival (OS). Neutrophil engraftment was defined as an absolute neutrophil count ≥0.5 × 10⁹/L for three consecutive days, and platelet engraftment was defined as ≥20 × 10⁹/L for seven consecutive days without platelet transfusion. aGVHD, cGVHD, NRM, relapse, LFS, and OS were defined as described previously. Patients with confirmed MRD were not classified as having a relapse.

Statistical analysis

Descriptive statistics, including the frequency (proportions) for categorical variables and the median (range) for
quantitative variables, were used to describe the patient demographic and clinical characteristics. LFS, OS, NRM, and relapse incidence curves were estimated with the Kaplan–Meier method. Separate analyses were performed for six landmarks, namely samples from days +30, +60, +90, +120, and +180 or more after allo-HSCT. In each landmark analysis, time was measured from the date of sampling. Cumulative incidence curves were used in a competing risk setting, with relapse treated as a competing event, to calculate NRM probabilities. Only variables with \( P < 0.1 \) were included in a Cox proportional hazards model with time-dependent variables. Only variables with \( P < 0.1 \) were included in a Cox proportional hazards model with time-dependent variables, while the \( P < 0.05 \) was defined as significant in the multivariate analysis.

Finally, the risk score system included three significant prognostic factors, disease status, post-MRD, and cGVHD status, which, based on the results of the multivariate analysis, would be established. This risk score system ranged from 0 to 3 (0 for no risk factor, 1 for any one of the three risk factors, 2 for any two of the three risk factors, and 3 for all three risk factors). Calculations were performed using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The characteristics and outcomes of the 477 patients with B-ALL who underwent allo-SCT are outlined in Table 1. There were 163 (34.2%) patients with positive Philadelphia chromosome (Ph) and 314 (65.8%) patients with negative Ph. The median age of the patients was 26 years, ranging from 2.5 to 63.0 years. Of all patients, 423 (88.7%) achieved CR1 before transplantation, and 54 (11.3%) achieved CR2 or CR3. There were 118 patients with pre-MRD+ and 77 patients with post-MRD+, and the subsequent pre-emptive interventions are shown in Figure 1.

By the end of the follow-up time, June 30, 2019, there was a median follow-up duration of 1570 (24–3107) days for all patients and 1816 (1277–3107) days for surviving patients; a total of 143 patients died, and the causes of death were relapse (53.8%), infection (28.7%), poor graft function (4.9%), GVHD (2.8%), and other causes (9.8%). All patients achieved neutrophil engraftment, and 95.4% of patients achieved platelet engraftment. The 100-day cumulative incidence of Grades III–IV aGVHD was 5.9% (95% confidence interval [CI] 3.7%–8.1%), while the 5-year cumulative incidence of cGVHD was 48% (95% CI 43.1%–52.9%); the transplant outcomes are listed in Table 2.

Variables associated with outcomes

Univariate analysis showed the following: (i) disease status (CR1 vs. CR ≥2) was related to CIR, LFS, and OS; (ii) pre-MRD (positive vs. negative), post-MRD (positive vs. negative), and cGVHD (yes or no) were associated with

Table 1: Demographic and clinical characteristics of patients with B-ALL underwent allo-SCT (n = 477).

| Parameters                  | Values |
|-----------------------------|--------|
| Age (years)                 | 26 (2.5–63.0) |
| Gender                      |        |
| Male                        | 267 (56.0) |
| Female                      | 210 (44.0) |
| ALL type                    |        |
| Ph positive                 | 163 (34.2) |
| Ph negative                 | 314 (65.8) |
| Disease status              |        |
| CR1                         | 423 (88.7) |
| CR ≥2                       | 54 (11.3) |
| Transplant type             |        |
| HBMT                        | 340 (71.3) |
| MSDT                        | 127 (26.6) |
| MUDT                        | 10 (2.1)  |
| Conditioning regimen        |        |
| MA                          | 477 (100) |
| Donor-recipient sex match   |        |
| Male-male                   | 170 (35.6) |
| Male-female                 | 130 (27.3) |
| Female-male                 | 96 (20.1)  |
| Female-female               | 81 (17.0)  |
| ABO matched                 |        |
| Matched                     | 259 (54.3) |
| Major mismatched            | 98 (20.5)  |
| Minor mismatched            | 93 (19.5)  |
| Bidirectional mismatched    | 27 (5.7)   |
| Infused cell doses           |        |
| MNC (\( \times 10^9\text{kg} \)) | 7.9 (2.5–20.1) |
| CD34+ cells (\( \times 10^9\text{kg} \)) | 2.5 (0.4–12.7) |
| Engraftment (yes or no)     |        |
| Neutrophil                  | 477 (100) |
| Platelet                    | 455 (95.4) |
| Engraftment (days)          |        |
| Neutrophil                  | 13 (9–26)  |
| Platelets                   | 14 (7–506)  |
| Acute GVHD grades           |        |
| I                           | 128 (26.8) |
| II                          | 95 (19.9)  |
| III                         | 19 (4.0)   |
| IV                          | 9 (1.9)    |
| Chronic GVHD (n = 197)      |        |
| Clinical extensive          | 44 (9.2)   |
| Median follow-up for surviving patients (days) | 1816 (1277–3107) |

Data are presented as median (range), or n (%). ALL: Acute lymphoblastic leukaemia; allo-SCT: Allogeneic stem cell transplantation; CR: Complete remission; CR1: First complete remission; GVHD: Graft-versus-host disease; HBMT: Haplodidentical blood and marrow transplantation; MA: Myeloablative conditioning regimen; MNC: Mononuclear cell; MSDT: MSDT: HLA-matched sibling donor transplantation; MUDT: HLA-matched unrelated donor transplantation; Ph: Philadelphia chromosome.

CIR, LFS, OS, and NRM; (iii) platelet engraftment (yes or no) and aGVHD III–IV (yes or no) were associated with LFS, OS, and NRM; while aGVHD (yes or no) was related to CIR. The multivariate analysis showed that patients with post-MRD+ suffered higher CIR (66.6% vs. 11.9%, \( P < 0.001 \)), lower LFS (28.2% vs. 72.8%, \( P < 0.001 \)), and OS (41.5% vs. 76.0%, \( P < 0.001 \)) than those with post-
Patients with post-MRD showed a trend of higher NRM than those with pre-MRD (5.2% vs. 1.5%, P = 0.057) in the univariate analysis, but there was no significance in the multivariate analysis. The landmark analysis showed that post-MRD at all time points (days +30, +60, +90, +120, and +180 or more) was inversely correlated with OS as well as LFS and positively correlated with CIR, but there was no relationship with NRM [Table 3].

In this study, a total of 77 patients had positive post-MRD, of which 60 were treated with pre-emptive interventions [Figure 1]. The CIR of the intervention group showed a lower incidence than that of the non-intervention group.

### Table 2: Transplant outcomes for B-ALL patients who underwent allo-SCT in different sub-group cases.

| Parameters | n   | 5-year relapse | 5-year OS | 5-year LFS | 5-year NRM |
|------------|-----|----------------|-----------|------------|------------|
| Total patients | 477 | 20.7 (17.0–24.4) | 70.4 (66.3–74.5) | 65.6 (61.3–69.9) | 13.9 (10.8–17.0) |
| Pre-MRD | | | | | |
| Negative | 359 | 15.5 (12.3–18.7) | 73.3 (68.6–78.0) | 69.0 (64.1–73.9) | 16.1 (12.1–20.1) |
| Positive | 118 | 36.4 (27.7–45.1) | 61.8 (53.0–70.6) | 55.3 (46.1–64.5) | 7.6 (2.8–12.4) |
| Post-MRD | | | | | |
| Negative | 400 | 11.9 (8.7–15.1) | 76.0 (71.7–80.3) | 72.8 (68.3–77.3) | 15.5 (11.9–19.1) |
| Positive | 77 | 66.6 (61.1–72.1) | 41.5 (52.5–30.5) | 28.2 (17.6–38.8) | 5.2 (0.2–10.2) |
| Risk score system | | | | | |
| 0 | 159 | 6.3 (2.6–10.0) | 85.7 (80.0–91.4) | 83.2 (77.1–89.3) | 10.6 (5.5–15.7) |
| 1 | 235 | 16.6 (11.7–21.5) | 69.2 (62.7–75.7) | 66.6 (60.5–72.7) | 17.1 (12.2–22.0) |
| 2 | 72 | 55.9 (43.9–67.5) | 36.6 (24.8–48.4) | 31.8 (20.8–42.8) | 12.3 (4.7–19.9) |
| 3 | 11 | 81.8 (55.3–100.0) | 27.3 (1.0–53.6) | 18.2 (0.2–40.9) | 0 |

Data are presented as percentage of incidence (95% confidence interval). *Indicates P < 0.05 compared with patients with pre-MRD positive. †Indicates P < 0.05 compared with patients with the other three risk scores. allo-SCT: Allogeneic stem cell transplantation; B-ALL: B cell acute lymphocytic leukemia; CI: Confidence interval; HR: Hazard ratio; LFS: Leukemia-free survival; MRD: Minimal residual disease; NRM: Non-relapse mortality; OS: Overall survival.
(68.1% vs. 75.2%), but the difference was not statistically significant (P = 0.394). Meanwhile, the OS (41.4% vs.
11.8%, P = 0.002) and LFS (31.9% vs. 15.7%, P = 0.039) were better and the TRM was lower (0% vs. 29.8%,
P < 0.001) in the intervention group than those in the non-intervention group.

The multivariate analysis also showed that higher disease status (≥CR2 vs. CR1) and the occurrence of cGVHD
(without cGVHD vs. with cGVHD) were associated with higher CIR (P = 0.004, P < 0.001), worse OS (P = 0.009, 
P < 0.001), and LFS (P = 0.017, P < 0.001). Patients without platelet (PLT) engraftment and with Grades III–
IV cGVHD had higher NRM (P < 0.001, P < 0.001), lower OS (P < 0.001, P = 0.005), and LFS (P < 0.001, 
P = 0.022) than patients with PLT engraftment and without Grades III–IV aGVHD.

**Improving relapse risk stratification with post-MRD, CR, and cGVHD status**

Considering the effects of disease status, post-MRD, and cGVHD on relapse, we performed sub-group analyses to
confirm whether the combination of the above risk factors could achieve further stratification of relapse and survival.

First, as shown in Table 4, the combination of CR and post-MRD status could stratify patients with different
incidences of relapse. For patients with post-MRD, there was a tendency for patients transplanted in ≥CR2 to have
a higher CIR than those in CR1 (80.0% vs. 63.3%, P = 0.096). Similar results were obtained in patients with
post-MRD, in which patients transplanted in ≥CR2 had a higher CIR than those transplanted in CR1 (25.3% vs.
10.5%, P = 0.007).

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### Table 3: Transplant outcomes for B-ALL patients at different time points after allo-SCT.

| MRD status at different time points | 5-year relapse | P value | 5-year OS | P value | 5-year LFS | P value | 5-year NRM | P value |
|------------------------------------|----------------|---------|-----------|---------|------------|---------|-----------|---------|
| +30 days                           | 21.7 (17.6–25.8) | <0.001  | 72.6 (68.5–76.7) | <0.001  | 67.7 (63.4–72.0) | 13.5 (10.4–16.6) | 0.416 |
| MRD−                               | 52.3 (28.2–76.4) | <0.001  | 36.8 (15.0–58.6) | <0.001  | 36.8 (15.0–58.6) | 18.9 (0–38.5) |
| +60 days                           | 21.6 (17.5–25.7) | <0.001  | 73.3 (69.2–77.4) | <0.001  | 68.3 (64.0–72.6) | 12.6 (9.5–15.7) |
| MRD+                               | 61.1 (35.8–86.4) | <0.001  | 33.3 (9.4–57.2)  | <0.001  | 33.3 (9.4–57.2)  | 11.1 (0–31.7)  |
| +90 days                           | 20.4 (16.5–24.3) | <0.001  | 75.4 (71.3–79.5) | 0.002   | 70.7 (66.4–75.0) | 11.0 (8.1–13.0) |
| MRD−                               | 70.0 (46.9–93.1) | <0.001  | 42.8 (18.5–67.1) | <0.001  | 30.0 (6.9–53.1)  | 0.230 |
| +120 days                          | 19.8 (15.9–23.7) | <0.001  | 76.8 (72.9–80.7) | <0.001  | 71.5 (67.2–75.8) | 10.7 (7.8–13.6) |
| MRD+                               | 73.3 (51.0–95.6) | <0.001  | 26.7 (4.4–49.0)  | <0.001  | 26.7 (4.4–49.0)  | 0.285 |
| +180 or more days                  | 13.9 (10.2–17.6) | <0.001  | 82.8 (79.1–86.0) | <0.001  | 79.1 (75.0–83.2) | 8.2 (5.5–10.9)  |
| MRD+                               | 75.0 (60.7–89.3) | <0.001  | 33.6 (14.8–52.4) | <0.001  | 25.0 (10.7–39.3) | 0.066 |

Data are presented as percentage of incidence (95% confidence interval). allo-SCT: Allogeneic stem cell transplantation; B-ALL: B cell acute lymphocytic leukemia; LFS: Leukemia-free survival; MRD: Minimal residual disease; NRM: Non-relapse mortality; OS: Overall survival.

### Table 4: The effects of pairwise combination of post-MRD, disease status, and cGVHD on transplant outcomes.

| Sub-groups | 5-year relapse | P value | 5-year OS | P value | 5-year LFS | P value | 5-year NRM | P value |
|------------|----------------|---------|-----------|---------|------------|---------|-----------|---------|
| Status of post-MRD and CR          |               |         |           |         |            |         |           |         |
| Post-MRD+ and CR1                   | 63.3 (50.4–76.2) | <0.001  | 36.5 (21.8–51.2) | <0.001  | 30.3 (18.3–42.3) | 6.5 (0.2–12.8) |
| Post-MRD+ and ≥CR2                   | 80.0 (57.7–100.0) | <0.001  | 25.0 (2.3–47.7)  | 0.007   | 20.0 (0–40.2)   | 0.002 |
| Post-MRD− and CR1                   | 10.5 (7.4–13.6)  | <0.001  | 77.5 (73.2–81.8) | <0.001  | 74.4 (69.9–78.9) | 14.9 (11.2–18.6) |
| Post-MRD− and ≥CR2                   | 25.3 (10.0–40.6) | <0.001  | 55.4 (37.6–73.2) | <0.001  | 56.8 (40.1–73.5) | 18.0 (5.8–30.2) |

Data are presented as percentage of incidence (95% confidence interval). aGVHD: Acute graft-versus-host disease; CI: Confidence interval; CR: Complete remission; LFS: Leukemia-free survival; MRD: Minimal residual disease; NRM: Non-relapse mortality; OS: Overall survival.
Second, we made four sub-groups based on the post-MRD and cGVHD status of all patients. As shown in Table 4, patients with post-MRD+ who developed cGVHD had a lower CIR than patients without cGVHD (6.3% vs. 16.3%, \(P < 0.001\)); meanwhile, patients with post-MRD+ who developed cGVHD had similar CIR compared to patients without cGVHD (61.3% vs. 69.2%, \(P = 0.159\)).

Third, the results of our study showed that patients transplanted in \(\geq\)CR2 had higher CIR than those in CR1, and further stratification analysis showed that patients transplanted in \(\geq\)CR2 who developed cGVHD could achieve the same relapse rate as patients transplanted in CR1 without cGVHD (21.1% vs. 23.7%, \(P = 0.631\)) [Table 4].

**Risk score for relapse in the entire cohort**

We chose three factors (including disease status, post-MRD status, and cGVHD status) to formulate a risk score according to the results of the multivariate analysis: 0 corresponded to patients in CR1, with post-MRD+ and with cGVHD, while 1 corresponded to patients in \(\geq\)CR2, with post-MRD+ but without cGVHD. All patients were divided into four sub-groups based on their score (0, 1, 2, or 3).

As expected, our study concluded that the 5-year CIR increased with increasing risk score: 6.3% (score 0), 16.6% (score 1), 53.9% (score 2), and 81.8% (score 3); the 5-year OS (85.7% vs. 69.2% vs. 36.6% vs. 27.3%) and 5-year LFS (83.2% vs. 66.6% vs. 31.8% vs. 18.2%) decreased with increasing risk score, and all relationships were statistically significant. Multivariate analysis showed that the risk score was an independent risk factor associated with CIR, LFS, and OS [Tables 2 and 5, and Figure 2].

**Discussion**

Our study analyzed the prognostic factors associated with transplant outcomes in patients with B-ALL who received allografts, and the results were as follows: (i) the factors associated with increased CIR and inferior survival were disease status, post-MRD, and cGVHD status; and (ii) a scoring system that could predict relapse and survival was...
established. These results added further evidence to the inferences that disease status, post-MRD status, and cGVHD status can predict relapse in patients with B-ALL receiving allo-SCT, and that the onset of cGVHD could improve the outcomes of patients in ≥CR2 and/or those with post-MRD. Moreover, the scoring system could stratify patients with different risks of relapse more precisely than using one indicator.

Consistent with previous studies, our study showed that patients with post-MRD suffered higher CIR and poorer survival than post-MRD patients. In addition, we also found that post-MRD at different time points was related to a higher incidence of relapse and a lower probability of LFS and OS, which was consistent with the results of Bader et al. The above results suggested that patients with positive post-MRD had a poor prognosis. Previous studies have illustrated that pre-emptive interventions (such as DLI and interferon) are effective treatments to reduce the CIR for patients with acute leukemia or myelodysplastic syndrome who become post-MRD positive after allo-SCT. Our study showed that pre-emptive interventions had a tendency to reduce the CIR for patients with acute GVHD, which was consistent with the results of Bader et al. The above results suggested that patients with positive post-MRD had a poor prognosis.

Table 5: Univariate and multivariate analysis of variables related to transplant outcomes among all patients.*

| Covariates                  | Univariate analysis | Multivariate analysis |
|-----------------------------|---------------------|-----------------------|
|                             | HR                  | 95% CI                | P value  | HR                  | 95% CI                | P value  |
| Relapse                     |                     |                       |         |                     |                       |         |
| Pre-MRD (positive vs. negative) | 2.724               | 1.824–4.068           | <0.001  | 1.576               | 1.031–2.409           | 0.036    |
| Risk score system 0         |                     |                       |         |                     |                       |         |
| 1                           | 3.348               | 1.616–6.938           | 0.001   | 3.062               | 1.525–6.148           | 0.002    |
| 2                           | 15.707              | 7.596–32.479          | <0.001  | 12.195              | 5.979–24.870          | <0.001   |
| 3                           | 31.916              | 12.598–80.854         | <0.001  | 25.356              | 10.135–63.435         | <0.001   |
| Acute GVHD (yes or no)      | 0.650               | 0.431–0.980           | 0.040   |                     |                       |         |
| LFS                         |                     |                       |         |                     |                       |         |
| Pre-MRD (positive vs. negative) | 1.605               | 1.153–2.234           | 0.005   |                     |                       |         |
| Risk score system 0         |                     |                       |         |                     |                       |         |
| 1                           | 2.411               | 1.533–3.792           | <0.001  | 2.094               | 1.335–3.284           | 0.001    |
| 2                           | 6.556               | 4.036–10.649          | <0.001  | 5.279               | 3.244–8.591           | <0.001   |
| 3                           | 10.246              | 4.765–22.031          | <0.001  | 11.070              | 5.158–23.761          | <0.001   |
| Platelet engraftment (yes or no) | 0.060               | 0.037–0.098           | <0.001  | 0.079               | 0.048–0.129           | <0.001   |
| Acute GVHD III–IV (yes or no) | 1.751               | 0.993–3.091           | 0.053   | 1.854               | 1.048–3.282           | 0.034    |
| NRM                         |                     |                       |         |                     |                       |         |
| Pre-MRD (positive vs. negative) | 0.507               | 0.251–1.026           | 0.059   |                     |                       |         |
| Risk score system 0         |                     |                       |         |                     |                       |         |
| 1                           | 1.856               | 1.037–3.322           | 0.037   |                     |                       |         |
| 2                           | 2.182               | 1.264–3.774           | <0.001  | 2.109               | 1.295–3.433           | 0.003    |
| 3                           | 3.433               | 2.014–5.883           | <0.001  | 3.118               | 1.880–5.127           | <0.001   |
| Platelet engraftment (yes or no) | 0.044               | 0.025–0.067           | <0.001  | 0.055               | 0.033–0.093           | <0.001   |
| Acute GVHD III–IV (yes or no) | 2.064               | 1.166–3.654           | 0.013   | 2.189               | 1.231–3.894           | 0.008    |

Results were calculated by Cox proportional hazards model. * All variables were included in the univariate analysis; only variables with P < 0.1 were included in the Cox proportional hazards model with time-dependent variables. CI: Confidence interval; GVHD: Chronic graft-versus-host disease; HR: Hazard ratio; LFS: Leukemia-free survival; MRD: Minimal residual disease; NRM: Non-relapse mortality; OS: Overall survival.
in our study: patients transplanted in ≥CR2 had a higher CIR than those in CR1. Moreover, patients transplanted in ≥CR2 were more likely to be post-MRD positive than those in CR1 (P = 0.016) in this study, which might be a reason why being in ≥CR2 was related to a higher CIR. Previous studies [26,32,37,38,40,41,66-68] also demonstrated that GVHD had a graft-versus-leukemia effect, but Grades III–IV aGVHD were accompanied by a higher NRM than cases without Grades III–IV aGVHD. [39,69] We also concluded that patients with Grades III–IV aGVHD had a higher NRM and poorer OS and LFS than those without Grades III–IV aGVHD, but there was no correlation with relapse. The results revealed that cGVHD instead of aGVHD could reduce the CIR and improve OS and LFS. A possible reason might be that the time of relapse for most patients was >100 days after transplantation, with a median time of relapse of 322 (45–1678) days after transplantation.

Considering the above results, we formulated a scoring system to predict transplant outcomes. The model consisted of three parameters: disease status, post-MRD status, and cGVHD status. The results indicated that the higher the score was, the higher the CIR, and the lower the LFS and OS, but there was no correlation with NRM [Figure 2]. Overall, all patients in this study had B-ALL, and the study contained a large sample size of patients undergoing HBMT. Some new insights can be drawn from these findings. First, the scoring system together with findings from our previous study[11] could help predict transplant outcomes precisely in ALL patients receiving allo-SCT. Second, for patients in ≥CR2 pre-transplant and/or with post-MRD*, we can reduce relapse by inducing cGVHD. However, it is important to undertake some caution in interpreting the scoring system. First, these data were derived from an allo-SCT setting, including HBMT, MSDT, and few MUDT cases, whereas further evidence under different transplant modalities is required to confirm the results. Second, we focused on total cGVHD in this study, but cGVHD may occur before, during, or after the post-MRD turns positive; as such, the effect of cGVHD occurring at different time points on outcomes needs to be further explored.

There are some limitations to this study. First, post-MRD was detected by MFC in this study, but specific biomarkers in patients with B-ALL, such as BCR/ABL, were not taken into account. Second, clinical intervention while MRD turned positive in some patients would affect clinical outcomes, which was an interfering factor for the risk score system. Finally, this was a single-center and retrospective study, and a multicenter study is needed to provide a large sample study with adequate statistical power.

In conclusion, our study indicated that disease status, post-MRD status, and cGVHD status were related to transplant outcomes in patients with B-ALL receiving allo-SCT. The most important accomplishment of this study was the formulation of a new scoring system with the three variables mentioned above; this scoring system could further stratify patients with different risks of relapse and guide treatment more precisely.

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**Conflicts of interest**

None.

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