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# The dynamics of B-cell reconstitution post allogeneic hematopoietic stem cell transplantation: A real-world study

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**Abstract.** Zhou G, Zhan Q, Huang L, Dou X, Cui J, Xiang L, et al. The dynamics of B-cell reconstitution post allogeneic hematopoietic stem cell transplantation: A real-world study. *J Intern Med.* 2024;00–00.

**Background.** The immune reconstitution after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is crucial for preventing infections and relapse and enhancing graft-versus-tumor effects. B cells play an important role in humoral immunity and immune regulation, but their reconstitution after allo-HSCT has not been well studied.

**Methods.** In this study, we analyzed the dynamics of B cells in 252 patients who underwent allo-HSCT for 2 years and assessed the impact of factors on B-cell reconstitution and their correlations with survival outcomes, as well as the development stages of B cells in the bone marrow and the subsets in the peripheral blood.

**Results.** We found that the B-cell reconstitution in the bone marrow was consistent with the peripheral blood ($p = 0.232$). B-cell reconstitution was delayed by the male gender, age >50, older donor age, the occurrence of chronic and acute graft-versus-host disease, and the infections of fungi and cytomegalovirus. The survival analysis revealed that patients with lower B cells had higher risks of death and relapse. More importantly, we used propensity score matching to obtain the conclusion that post-1-year B-cell reconstitution is better in females. Meanwhile, using mediation analysis, we proposed the age-B cells-survival axis and found that B-cell reconstitution at month 12 posttransplant mediated the effect of age on patient survival ($p = 0.013$). We also found that younger patients showed more immature B cells in the bone marrow after transplantation ($p = 0.037$).

**Conclusion.** Our findings provide valuable insights for optimizing the management of B-cell reconstitution and improving the efficacy and safety of allo-HSCT.

**Keywords:** allo-HSCT, B cells, immune reconstitution, mediation analysis, propensity score matching

## Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a crucial modality for the treatment of malignant and benign diseases of the hematological system [1, 2]. Post-HSCT immune reconstitution is the process of restoring the normal function and diversity of the immune system after HSCT and is an important factor affecting the outcomes and complications of HSCT [3].

Guangyu Zhou and Qian Zhan have contributed equally to this work and shared first authorship.

Previous studies have shown that immune reconstitution is a dynamic process that involves different subpopulations of immune cells, each with its own reconstitution pattern and kinetics. Moreover, immune reconstitution varies greatly among individuals and is influenced by multiple factors related to the donor, the recipient, and the transplantation procedure [4–8]. Most of the previous studies have focused on the reconstitution of T cells and natural killer (NK) cells, which play key roles in cellular immunity [9–11]. A previous study showed that the slow reconstitution of B cells is one of the reasons why patients are susceptible to infections after transplantation [12]. However, the dynamics and regulation of B-cell reconstitution, which is essential for immune tolerance, have not been well studied. Furthermore, the relationship between B-cell reconstitution and the prognosis of allo-HSCT recipients remains unclear and needs further exploration.

Understanding B-cell reconstitution is essential for gaining a more comprehensive insight into immune reconstitution after HSCT, as B cells are responsible for producing antibodies and regulating immune responses in the adaptive immune system [13, 14]. After hematopoietic stem cell transplantation, B cells undergo a process of recovery to normal levels, not only in terms of quantity but also in terms of phenotype [15, 16]. To explore the factors that affect B-cell reconstitution after transplantation and the impact of B-cell recovery on patient outcomes, we creatively analyzed the changes of B cells in the bone marrow and combined them with the subpopulations of peripheral blood, which vividly revealed the dynamics of B-cell reconstitution. The propensity score matching (PSM) eliminates the confounding factors associated with retrospective studies. Mediation analysis is an emerging and widely used method for addressing various clinical problems [17, 18], which inspired us to study the role of B cells in the transplantation process.

In this study, we aimed at investigating the kinetics of B-cell reconstitution in 252 patients who underwent allo-HSCT and to analyze the impact of various factors on B-cell recovery and its association with survival outcomes (Fig. 1). We revealed for the first time the relationship between B-cell reconstitution and gender and innovatively proposed the age-B cells-survival axis. Our findings provide valuable insights for optimizing the management of B-cell reconstitution and improving the efficacy and safety of allo-HSCT.

## Patients and methods

### *Patients*

We conducted a retrospective cohort study of patients who underwent allo-HSCT at the Department of Hematology, the First Affiliated Hospital of Chongqing Medical University, from January 2016 to June 2022. The study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University and followed the Declaration of Helsinki. The cohort included 252 patients. Informed consent was obtained from each participant.

The data for the outcome were collected by retrieving information registered by the Electronic Hospital Record system and/or by phone-contacting patients. Considering the first day of cell transfusion as day 0, B-cell data were collected at the 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 12th, 18th, and 24th months after transplantation. For the peripheral blood B-cell subpopulation analysis, we randomly selected 10 patients, and 10 healthy controls, who were used for comparison, were age- and sex-matched with the 10 patients (Table S1)

Myeloablative conditioning (MAC) is defined as the chemotherapy regimen based on sufficient dose of busulfan and cyclophosphamide for myeloablative effects as determined by the clinician; reduced intensity conditioning (RIC) is defined as the chemotherapy regimen in which fludarabine or cladribine is substituted for cyclophosphamide, thereby reducing the toxicity of cyclophosphamide. The selection of preconditioning regimen was based on the Consensus of Chinese Hematopoietic Stem Cell Transplantation Experts (Table S2). Viral infection is defined as the presence of Epstein–Barr virus, cytomegalovirus (CMV), or herpes simplex virus infection detected by DNA copies between the transplant and the final follow-up time. Prophylactic viral and fungal drugs during transplantation were carried out according to the Chinese Invasive Fungal Infection Working Group, Society of Hematology, Diagnostic criteria and treatment principles of invasive fungal disease in patients with hematological diseases/malignant tumors (the fifth and sixth revisions), the sixth European Conference on Infections in Leukemia guidelines, and the NCCN

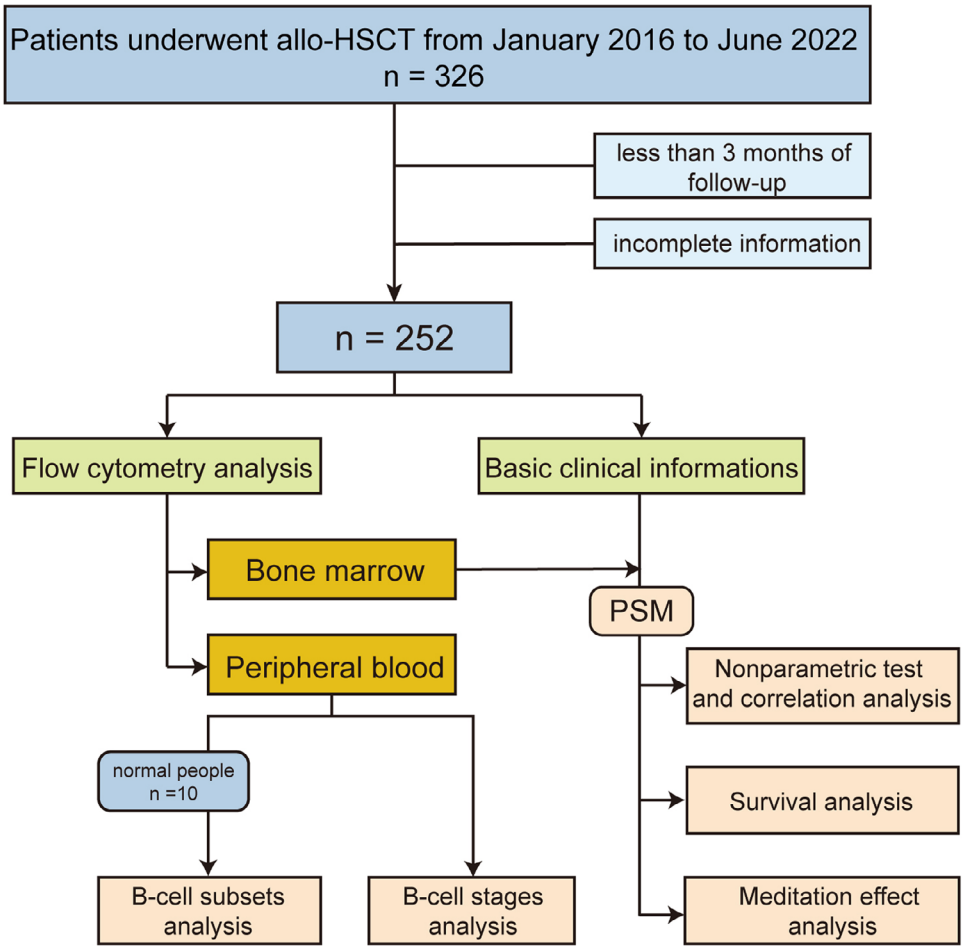


**Fig. 1** *Flowchart of this study.*

Guidelines in hematopoietic cell transplantation formulation [19–22]. We defined acute graft-versus-host disease (aGVHD) and chronic GVHD (cGVHD) according to the clinical diagnosis and grading by the attending physician, following the NIH criteria [23]. GVHD prophylaxis included cyclosporine A (CsA 3.0 mg/kg/day intravenously starting from day −1, adjusted to a trough level between 200 and 300 ng/mL [24]) +methotrexate (MTX 15 mg/m$^2$ intravenously on day +1 and 10 mg/m$^2$ on days +3, +6, and +11 [25]) +mycophenolate mofetil (MMF 0.5–1.0 g daily for 30 days [26]), and tacrolimus (FK506 0.05 mg/kg twice daily intravenously, adjusted to a trough level between 5 and 15 ng/mL [27]) + MTX + MMF. Thymoglobulin (ATG; Sanofi, divided into 3 days intravenous infusion [28]) is used in some patients.

*Flow cytometric cell analysis*

B-cell immune reconstitution of allo-HSCT patients was analyzed using existing bone marrow flow cytometry data. The patients underwent routine bone marrow flow cytometry follow-up for relapse or minimal residual disease detection until 2 years after HSCT.

Bone marrow samples were stained with CD3 FITC/CD16+56 PE/CD45 PerCP CY5.5/CD4 PC7/CD19 APC/CD8 APC-CY7 antibodies and examined with a decolor flow cytometer (Beckman NAVIOS) at the Center for Clinical Molecular Medical detection of the First Affiliated Hospital of Chongqing Medical University. Peripheral blood samples from the recipients were examined by flow cytometer (BD Canto II and Beckman NAVIOS), stained with IgD FITC/CD27 PE/CD14 ECD/CD3

PC5.5/CD19 PC7/CD24 APC/CD20 A750/CD38 V450/CD45KO. Data were analyzed using Kaluza software.

In the bone marrow, lymphocytes were defined as cells within the forward/sideward scatter lymphocyte gate. B cells were defined as CD19+ cells in the lymphocyte gate, which were calculated as the percentage of CD19+ cells to all nucleated cells (Fig. S1a–f). B-cell development is divided into three stages: Progenitor B cells, precursor B cells, and immature B cells. The three stages are defined as three groups of cells in the B lymphocyte gate (Fig. S1h).

In peripheral blood (Fig. S2), memory B cells as CD19+CD27+ cells within the lymphocyte gate are calculated as a percentage of CD19+ B cells. Transitional B cells (Tr B) as CD19+CD24++ CD38++(CD27−IgM+) cells within the lymphocyte gate are calculated as a percentage of CD19+ B cells. Marginal Zone B cells as CD19+IgD+CD27+ cells within the lymphocyte gate are calculated as a percentage of CD19+ B cells. B naïve as CD19+IgD+CD27-cells within the lymphocyte gate are calculated as a percentage of CD19+ B cells.

### *Statistical analysis*

Nonparametric tests were used to compare the number of B cells at different time points, as the data did not meet the assumptions of normality and homogeneity of variance. The chi-square test was used to verify whether there is a significant difference between B cells in peripheral blood and bone marrow samples. The Wilcoxon rank test or Kruskal–Wallis test was applied to assess differences in B-cell percentages between groups for categorical variables. Pearson or Spearman correlation analyses were performed to examine the relationship between each variable and B cell at different times for continuous variables. Independent samples *t*-tests were used to validate differences in B-cell subpopulations between normal subjects and patients. Comparisons between groups were made with the chi-square test. PSM was based on the R software package "MatchIt."

Relapse-free survival (RFS) was measured as the time from transplantation to relapse, and overall survival (OS) as the time from transplantation to the last follow-up or death. Log-rank test was used to determine the optimal cutoff values of B cells at different time points for predicting death and relapse, respectively. These cutoff values were used to classify the B cells into high and low groups at each time point. The survival curves between the high and low B-cell groups were then compared using the Kaplan–Meier method. The Cox proportional hazards regression models were performed to identify the risk factors for survival and relapse after transplantation. Survival analysis was conducted by using R software packages "survival," "survminer," "ggpubr," and "ggsci."

The causal mediation analysis based on the counterfactual framework was used to test whether B-cell immune reconstitution mediated the effect of age on posttransplant survival [29]. This analysis decomposed the total effect of an exposure (age) on an outcome (survival) into a direct effect and an indirect effect through a mediator variable (B cells). The direct and indirect effects and their 95% confidence intervals were estimated using quasi–Bayesian methods with the R software package "mediation". A sensitivity analysis was also conducted to assess the impact of potential unmeasured confounders on the mediation results [30]. The relationship among age, B cells, and survival status was visualized using Sankey plots [31] (https://www.sankeymatic.com).

All statistical analyses were performed using SPSS software (R26.0.0.0) and R software version 4.2.3. *p*-Values less than 0.05 were considered statistically significant.

## Results

### *Patients characteristics*

We enrolled 252 patients in our study, of whom 23% were older than 50 years (Table 1). The majority of the patients had acute leukemia and received transplantation in the first complete remission (82.5%). The donors were predominantly haploidentical (65.9%), with a median age of 42 years. Peripheral blood stem cells were the main source of grafts (97.6%). The aGVHD occurred in 46 patients, whereas cGVHD occurred in 60 patients. The median follow-up time was 23.32 months. Thirty-five patients experienced relapse, and 61 patients died.

### *B-cell reconstitution in bone marrow and peripheral blood*

We analyzed the flow cytometry data of bone marrow and peripheral blood samples to examine the

**Table 1.** *Basic characteristics of patients.*

| Characteristics | | $n = 252$ | % |
|---|---|---|---|
| Age at HSCT (years) | | | |
| | Median (range) | 33.5 (9, 65) | |
| | <50 | 194 | 77.00 |
| | >50 | 58 | 23.00 |
| Sex | Male | 137 | 54.4 |
| | Female | 115 | 45.6 |
| Diagnosis | ALL | 84 | 33.30 |
| | AML | 129 | 51.20 |
| | CML | 8 | 3.20 |
| | Lymphoma | 6 | 2.40 |
| | MDS | 20 | 7.90 |
| | MM | 5 | 2.00 |
| Status at HSCT | CR1/steady | 208 | 82.5 |
| | CR2 | 10 | 4 |
| | PR | 8 | 3.2 |
| | NR | 26 | 10.3 |
| Donor | MRD | 65 | 25.8 |
| | MMRD/haplo-identical | 166 | 65.9 |
| | MMUD | 7 | 2.8 |
| | MUD | 14 | 5.6 |
| Donor age | Median (range) | 42 (9, 65) | |
| Donor sex | Male | 68 | 27 |
| | Female | 184 | 73 |
| HLA match | Median (range) | 7 (3,12) | |
| Conditioning | MAC | 237 | 94 |
| | RIC | 15 | 6 |
| Use of ATG | | 169 | 67.1 |
| Source of stem cells | BM | 1 | 0.4 |
| | PB | 246 | 97.6 |
| | PB+BM | 6 | 2 |
| MNC dose (*10^8/kg) | Median (range) | 14.24 (2.34, 39.3) | |
| CD34+cell dose (*10^6/kg) | Median (range) | 6.38 (1.56, 23.91) | |
| GVHD prophylaxis | CsA+MMF+MTX | 192 | 76.2 |
| | FK506+MMF+MTX | 60 | 23.8 |
| aGVHD | | 46 | 18.30 |
| cGVHD | | 60 | 23.80 |
| Virus infections | Infected | 140 | 55.6 |
| | CMV | 102 | |
| | EBV | 81 | |
| | HSV | 57 | |
| Bacteria infections | | 233 | 92.5 |

(*Continued*)

**Table 1.** *(Continued)*

| Characteristics | | $n = 252$ | % |
|---|---|---|---|
| Fungi infections | | 118 | 46.8 |
| Follow-up (months) | Median (range) | 23.32 (1, 85.63) | |
| Relapse | | 35 | 13.9 |
| Death | | 61 | 24.2 |

Abbreviations: aGVHD, aacute graft-versus-host disease; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CML, chronic myeloid leukemia; CR, complete remission; HSV, herpes simplex virus; PR, partial remission; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MM, multiple myeloma; MMF, mycophenolate mofetil; MRD, minimal residual disease; MTX, methotrexate.; NR, non-remission; PB, peripheral blood; RIC, reduced intensity conditioning.

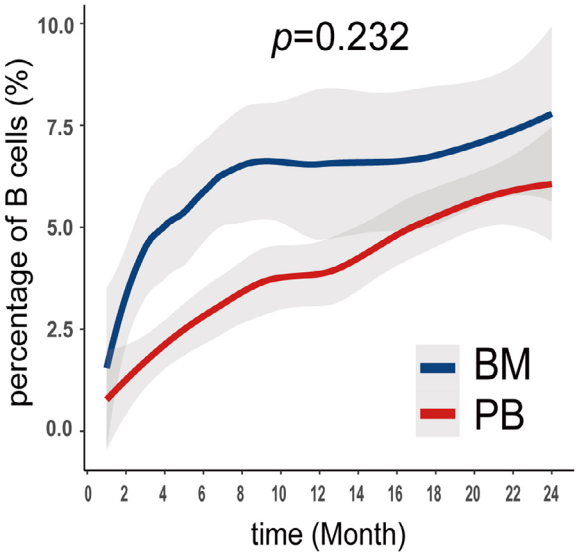


**Fig. 2** *The dynamics of B-cell counts in peripheral blood (PB) and bone marrow (BM) from months 1 to 24 posttransplantation.*

B-cell reconstitution curve over 2-year posttransplantation. B cells increased until 1 year after transplantation and then reached a plateau. By chi-square test, the B-cell reconstitution was consistent ($p = 0.232$) between bone marrow and peripheral blood (Fig. 2).

### *Factors influencing B-cell reconstitution*

We used nonparametric tests to compare the B-cell frequency among subgroups at different time points (Fig. 3a–i) (Table S3). We found that the B-cell frequency was higher in the CsA group than in the tacrolimus group at 1 month after transplantation but lower in the CsA group than in the tacrolimus group subsequently. Fungal infections during transplantation impaired B-cell reconstitution at 3 and 6 months after transplantation. B-cell reconstitution was also compromised in patients older than 50 years. Female patients had higher B-cell frequency than male patients in the late posttransplant period (from 12 to 24 months). Patients who developed aGVHD had lower B-cell frequency than those who did not at 2 and 3 months after transplantation. Patients who developed cGVHD had lower B-cell frequency than those who did not at 8 and 18 months after transplantation. CMV infection also adversely affected B-cell reconstitution at 6 months after transplantation. Patients who received the MAC regimen had better B-cell reconstitution than those who received the RIC regimen. Better B-cell reconstitution was associated with improved survival, as the survivors had significantly higher B-cell frequency than the nonsurvivors at various time points posttransplant.

To examine the relationship between B cells and continuous variables, we performed a correlation analysis (Fig. 3j–l) (Table S4). We observed significant negative correlations between B cells and both recipient and donor age, indicating that older age delayed B-cell reconstitution. We also found positive correlations between B cells and infused CD34+ cell count at 2 and 4 months after transplantation. HLA compatibility and infused MNC did not show significant correlations with B cells.

### *Effect of B-cell reconstitution on prognosis*

We performed a survival analysis to investigate the relationship between patient relapse or mortality and B-cell reconstitution. We divided B cells into high and low groups at each time point (Table S5). The Cox proportional hazards regression model revealed that better B-cell reconstitution at 3 and 12 months after transplantation was associated

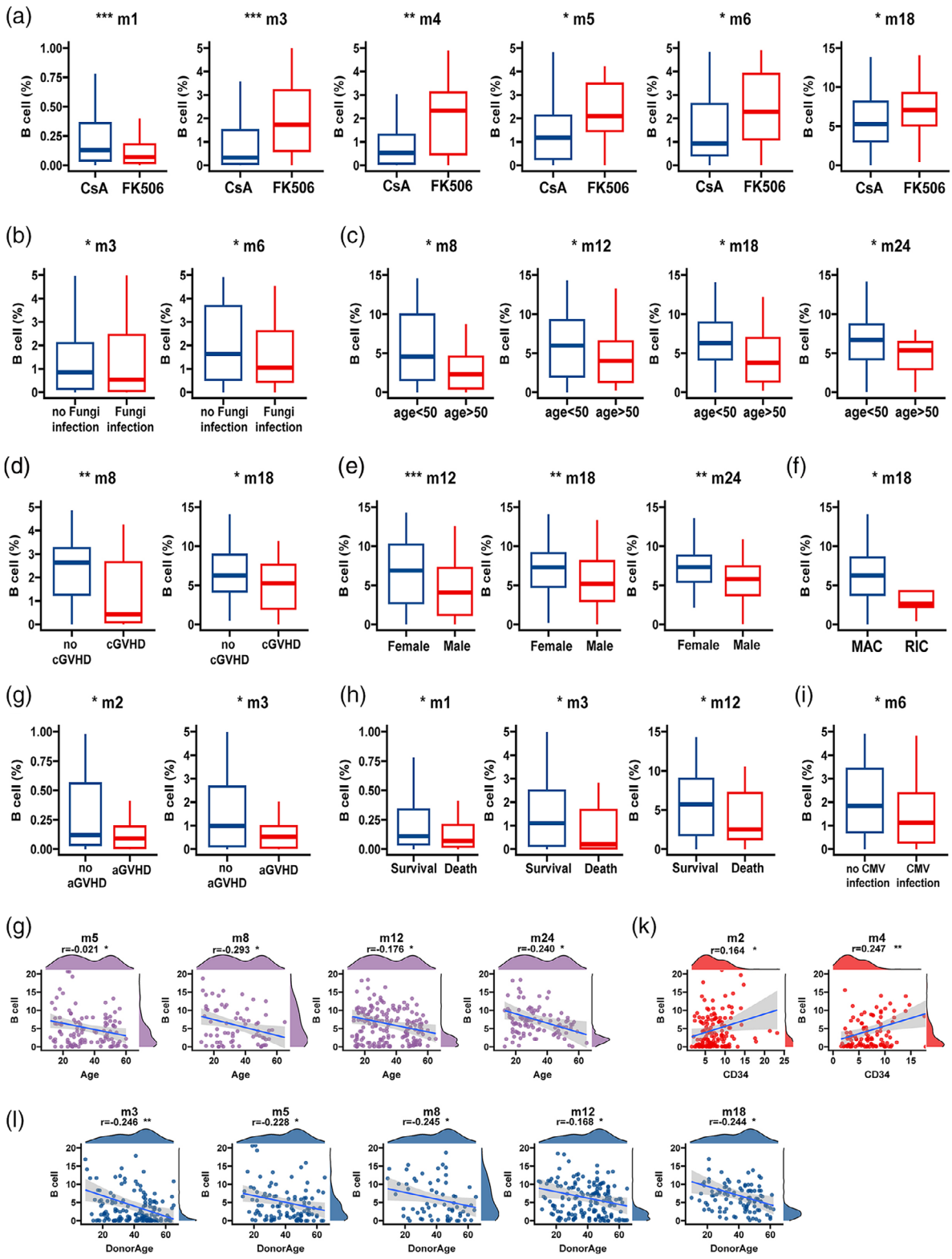

**Table 2.** *Cox proportional hazards regression model for prediction overall survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT).*

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Variables | HR (95%CI) | *p*-Value | HR (95%CI) | *p*-Value |
| Sex male | 1.697 (0.9722, 2.964) | 0.063 | 1.5364 (0.8192, 2.8814) | 0.181 |
| RIC | 2.534 (1.079, 5.949) | 0.033 | 1.7286 (0.6771, 4.413) | 0.252 |
| FK506 | 0.532 (0.2928,0.9665) | 0.038 | 0.678 (0.361, 1.2733) | 0.227 |
| Donor sex male | 0.4654 (0.2683, 0.8074) | 0.007 | 0.51671 (0.2916, 0.9157) | 0.024 |
| Age >50 | 2.491 (1.426, 4.351) | 0.001 | 2.3904 (1.3299, 4.2965) | 0.004 |
| m1 B-cell high | 0.5614 (0.323, 0.9759) | 0.041 | 0.65965 (0.3686, 1.1803) | 0.161 |
| m2 B-cell high | 0.5318 (0.2682, 1.055) | 0.071 | 0.65425 (0.3635, 1.1774) | 0.157 |
| m3 B-cell high | 0.2841 (0.1445, 0.5583) | 0.000 | 0.45729 (0.2506, 0.8345) | 0.011 |
| m4 B-cell high | 0.4495 (0.1844, 1.096) | 0.079 | 1.04176 (0.518, 2.0953) | 0.909 |
| m6 B-cell high | 0.2978 (0.13, 0.6822) | 0.004 | 0.84557 (0.4349, 1.644) | 0.621 |
| m12 B-cell high | 0.3445 (0.1531, 0.7753) | 0.010 | 0.43599 (0.2313, 0.8219) | 0.010 |
| m18 B-cell high | 0.2617 (0.07988, 0.8575) | 0.027 | 0.79401 (0.3932, 1.6034) | 0.520 |
| m24 B-cell high | 0.1464 (0.03385, 0.6333) | 0.010 | 1.59289 (0.7604, 3.3369) | 0.217 |

Abbreviations: CI, confidence interval; HR, hazard ratio; RIC, reduced intensity conditioning.

**Table 3.** *Cox proportional hazards regression model for prediction relapse-free survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT).*

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Variables | HR (95%CI) | *p*-Value | HR (95%CI) | *p*-Value |
| Sex male | 0.8267 (0.422, 1.619) | 0.579 | | |
| FK506 | 1.862 (0.9507, 3.648) | 0.070 | 1.48651 (0.73386, 3.011) | 0.271 |
| cGVHD | 0.08845 (0.0121, 0.6468) | 0.017 | 0.084987 (0.01137, 0.6353) | 0.016 |
| status at CR2 | 3.9405 (1.3744, 11.298) | 0.011 | 3.60257 (1.22158, 10.6243) | 0.020 |
| Age >50 | 0.8832 (0.3846, 2.028) | 0.770 | | |
| m1 B-cell high | 0.53 (0.2617, 1.073) | 0.078 | 0.63778 (0.31087, 1.3085) | 0.220 |
| m18 B-cell high | 2.879 (0.8783, 9.438) | 0.081 | 0.67223 (0.22988, 1.9658) | 0.468 |
| m24 B-cell high | 0.2022 (0.05218, 0.7834) | 0.021 | 0.74591 (0.31718, 1.7542) | 0.502 |

Abbreviations: cGVHD, chronic graft-versus-host disease; CI, confidence interval; HR, hazard ratio.

with improved survival (Table 2). Factors that reduced the risk of death (HR < 1) were better B-cell reconstitution and male donors, whereas age >50 years increased the risk of death. Likewise, the occurrence of cGVHD reduces the risk of relapse (Table 3). We also plotted the Kaplan–Meier curves for the high and low B-cell groups at different time points and found that the high B-cell group had significantly better OS (Fig. 4a) and RFS (Fig. 4b) than the low B-cell group.

### *Effect of gender on B-cell reconstitution after PSM*

In our study, we found that gender has an effect on post 1-year B-cell reconstitution, which was shown to be better in females than in males

**Fig. 3** *Correlation between B-cell reconstitution and variables: (a) The boxplot of B-cell proportions by graft-versus-host disease (GVHD) prophylaxis regimen; (b) the boxplot of B-cell proportions by fungal infections; (c) the boxplot of B-cell proportions by age; (d) the boxplot of B-cell proportions by chronic GVHD (cGVHD); (e) the boxplot of B-cell proportions by gender; (f) the boxplot of B-cell proportions by condition; (g) the boxplot of B-cell proportions by acute GVHD (aGVHD); (h) the boxplot of B-cell proportions by survival; (i) the boxplot of B-cell proportions by cytomegalovirus (CMV) infections; (j) the correlation fitting plot of B-cell percentage and patient age; (k) the correlation fitting plot of B-cell percentage and CD34+ cell numbers; and (l) the correlation fitting plot of B-cell percentage and donor age. *p < 0.05, **p < 0.01, and ***p < 0.001.*

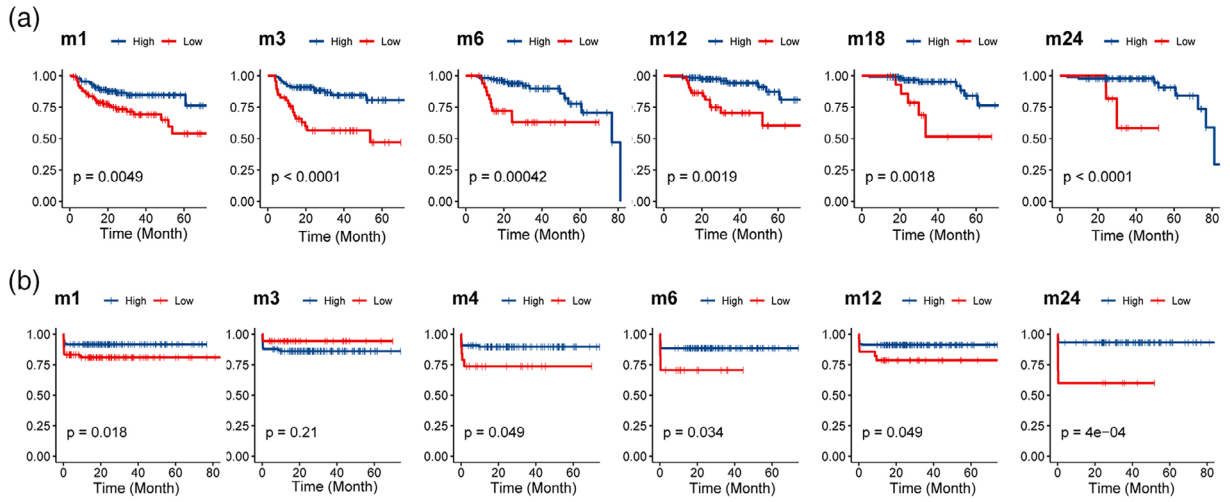


**Fig. 4** *The Kaplan–Meier curves of patients divided into B cells high and low groups: (a) the Kaplan–Meier curves of overall survival at different time points; (b) the Kaplan–Meier curves of progression-free survival at different time points; the blue line indicates the high group of B cells, the red line indicates the low group of B cells.*

(Fig. 3e). The PSM was used to exclude the influence of confounding factors other than gender on the final results. Grouping by sex (Table S6), there was a significant difference in cGVHD in the raw data ($p = 0.013$). With PSM (Fig. S3), we included the confounding factor, and matched a cohort of 69 pairs, and once again performed the chi-square test we found that the difference between the groups had been eliminated. Based on the matched cohort, the nonparametric test again demonstrated that long-term B-cell reconstitution was superior in women than men with confounding factors were excluded (Fig. 5a).

To further explore the effect of gender with respect to posttransplant month 12 B-cell and cGVHD differences, we used Sankey plots (Fig. 5b) to visualize the relationships. We found that women had better reconstitution and that good B-cell reconstitution was a protective factor for the development of cGVHD. In contrast, men had poor B-cell reconstitution at month 12 posttransplant and were more likely to develop cGVHD with poor B-cell reconstitution.

### *Age-B cells-survival axis*

We found that age >50 was significantly associated with poor B-cell reconstitution at the 12th month after transplantation and that deceased patients had poor B-cell reconstitution at the 12th month after transplantation. In the Cox proportional hazards regression model, age >50 was a key factor that independently influenced patients' survival. These results suggested a potential link among age, B cells, and survival. To test this hypothesis, we performed a mediation analysis and estimated the direct and indirect effects (Table S7 and Fig. S4). We revealed a causal pathway among the three variables (Fig. 6a): The frequency of B cells at 12 months posttransplant mediated the effect of age on survival ($p = 0.013$), accounting for 14.71% of the total effect. Moreover, we confirmed a direct effect of age on survival ($p = 0.04$).

Among transplant patients under the age of 50, the majority of patients had good B-cell reconstitution at the 12th month after transplantation (Fig. 6b). However, in elderly patients, about 1/3 of patients ($n = 21$) had poor B-cell reconstitution. There were 19 deaths from the B-cell low group and only 12 from the B-cell high group.

### *B-cell stages and subsets*

We observed that aging impairs the recovery of CD19+ B cells in the bone marrow, as evidenced by their low frequency. To elucidate their developmental dynamics, we performed a cross-sectional analysis of bone marrow B cells of 161 patients at the 1-year posttransplantation time point (Fig. 7a). We found that the proportion of immature B cells, the final stage of B-cell development in the bone marrow, was significantly lower in patients older

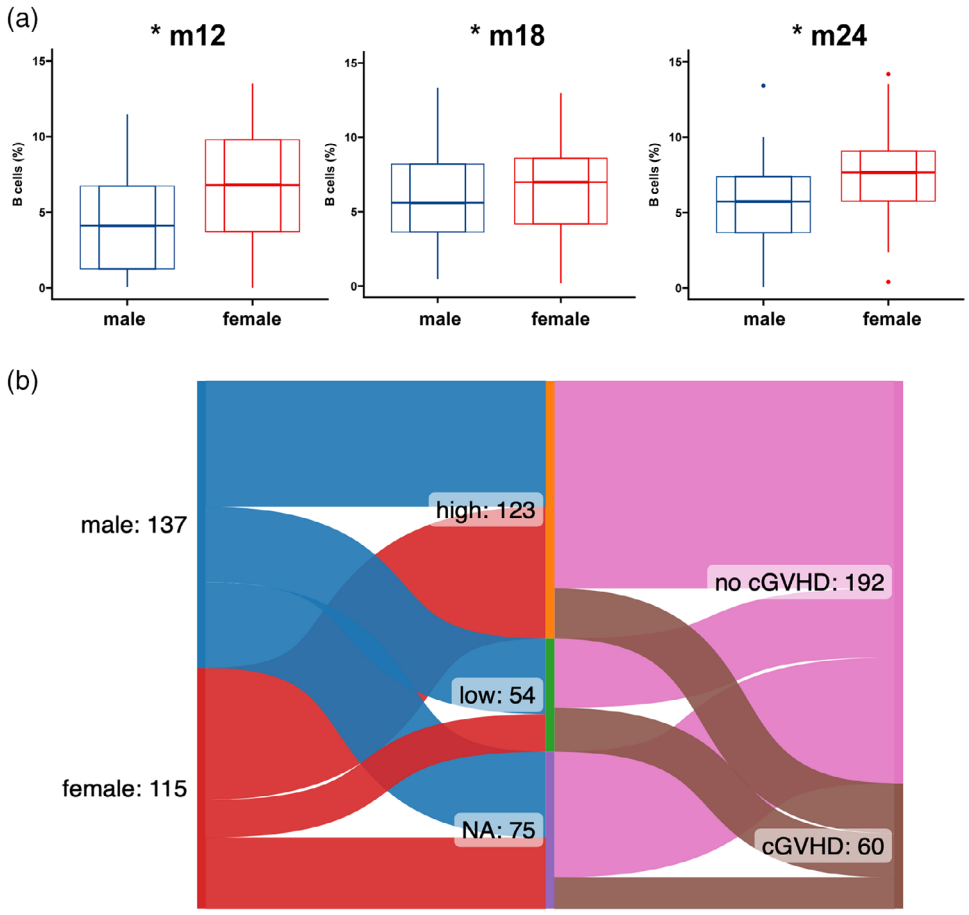


**Fig. 5** *Analysis of B-cell reconstitution grouped by sex: (a) differences in B-cell reconstitution at month 12, 18, and 24 between male and female groups after propensity score matching (PSM), *p < 0.05; (b) Sankey diagram of the relationship between sex-B cell-chronic graft-versus-host disease (cGVHD).*

than 50 years compared to those younger than 50 years ($p = 0.037$).

We analyzed the peripheral blood B-cell subsets of 10 patients at 12 months after transplantation (Fig. 7b) and found that naïve B cells (63.61%) were the predominant population of peripheral blood B cells, followed by memory B cells. Compared to 10 healthy controls, we observed that the frequencies of CD19+ and CD20+ cells were higher in the patients ($p < 0.001$), whereas the frequency of plasma cells was lower in the patients ($p = 0.019$). Fig. 7c shows that patients older than 50 years had lower frequencies of naïve and Tr B cells in the peripheral blood at 12 months after transplantation than the younger group, whereas memory B cells were much scarcer in the younger group than in the older group.

## Discussion

In this study, we conducted a retrospective analysis of B-cell immune reconstitution and its associated factors and outcomes in 252 patients who received allo-HSCT. The results showed that post-transplant B-cell immune reconstitution was influenced by various factors. In addition, impaired B-cell immune reconstitution was associated with an increased risk of relapse and mortality.

Our findings are in agreement with previous studies that reported heterogeneous and delayed B-cell reconstitution after allo-HSCT and its association with different patients, donor, and treatment-related factors. Because we included all patients who underwent HSCT, disease heterogeneity may have had an impact on the results. We distinguish

 

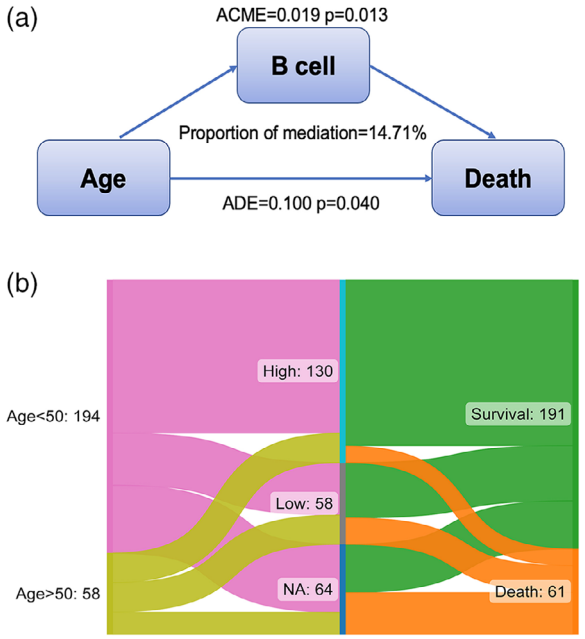


**Fig. 6** *The relationship among age, B cells, and survival: (a) age-B cells-survival axis. Proportion of B-cell mediation effect and (b) Sankey diagram of age-B cells-survival axis.*

between diseases (AML, MDS, and chronic myeloid leukemia [CML] = Group A, myeloid malignancies) and (ALL, MM, and lymphoma = Group B, malignancies), to evaluate and describe the demographic characteristics of the patients in each group (Table S8–S9 and Fig. S5). The results were consistent with the main analysis. However, there was a significant difference between the two groups in the age at transplantation, which could be explained by the fact that the incidence of AML increases with age, whereas all is mainly concentrated in young people and adolescents.

We noted that female recipients had higher B cells than male recipients in the late posttransplant period, which might be related to the hormonal effects on B-cell development and function [32, 33]. However, premature ovarian failure occurs in female after hematopoietic stem cell transplantation, and thus hormone level changes have not been systematically studied, so the mechanism of better B-cell reconstitution in female is not clear, and further prospective studies may be needed. In addition, we found that both recipient and donor age negatively affected B-cell reconstitution. This may indicate that thymic function and bone marrow activity deteriorate with aging, which impairs

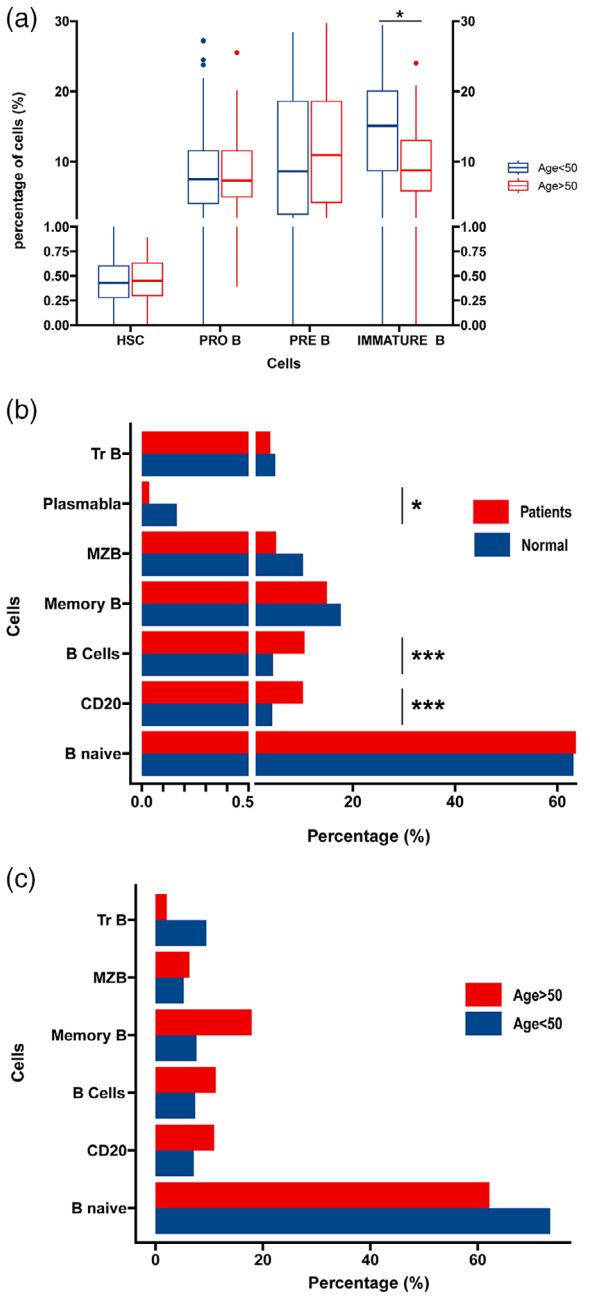


**Fig. 7** *The stages and subsets of B cells: (a) the percentage of different stages of B cells and hematopoietic stem cells (HSCs) in the bone marrow of old and young patients at 12 months after transplantation and comparison; (b) the percentage of B-cell subsets in the peripheral blood of patients at 12 months after transplantation and comparison with normal people; (c) the comparison of B-cell subsets in the peripheral blood of elderly and young patients at 12 months after transplantation.*

the production of naïve and mature B cells [34–37]. However, further studies are needed to elucidate the mechanisms of delayed B-cell immune reconstitution associated with aging. We analyzed the effects of different GVHD prophylaxis regimens on B-cell reconstitution and found that the FK506 regimen was associated with higher B cells with the CsA-based regimen (Table S10 and Fig. S6). B-cell growth and antibody production are indirectly affected by the inhibition of T cell-derived growth factor, which is essential for these functions, so the suppression of B cells by T cells may be attenuated in the FK506 group [38]. Stevens et al. demonstrated a direct inhibitory effect of CsA human B cells in response to IL-6. In contrast, FK506 had no direct effect on these B-cell lines [39]. We also analyzed the effect of different intensity pretreatment regimens on posttransplant B-cell reconstitution and found that patients using the MAC regimen instead had better reconstitution. This may be related to the fact that RIC is mainly used in elderly patients, and this group of patients is affected by age and delays B-cell reconstitution. This is also in line with the findings of previous studies [40]. The previous study mentioned that ATG use delayed B-cell reconstitution [41], but no effect of ATG use on B-cell reconstitution was observed in our analyses, which may be a result due to the heterogeneity among the cohorts. Because ATG mainly affects T cells, its mechanism of action on posttransplantation B cells till needs to be further investigated. CMV infection was associated with poor B-cell reconstitution at 6 and 18 months after transplantation. In patients who have undergone HSCT, latent CMV is of 10 reactivated in the early stages of immune recovery, usually within 3–4 months after HSCT, so it is more likely that CMV infection delays the reconstitution of B cells [42]. This is a very interesting finding of the study. It has been suggested that CMV infection drives or at least exacerbates “immunosenescence” [43]. However, it is not clear whether CMV infection directly affects immune recovery, which is worthy of further exploration from the mechanism and clinical.

Hematopoiesis recovery and immune reconstitution are two interrelated processes that depend on various factors. Generally, hematopoiesis recovery occurs faster than immune reconstitution, as the transplanted stem cells can differentiate into various blood cell lineages, such as erythrocytes, platelets, granulocytes, and monocytes, within weeks after HSCT [44]. Immune reconstitution can be influenced by hematopoiesis recovery, as the different blood cell lineages can interact with each other and modulate the immune response. For example, granulocytes and monocytes can provide innate immunity and cytokine production, whereas erythrocytes and platelets can affect the trafficking and activation of lymphocytes [45–47]. Therefore, hematopoiesis recovery and immune reconstitution are closely related, but not necessarily correlated, processes that occur after HSCT. The optimal balance between hematopoiesis recovery and immune reconstitution is crucial for the success of HSCT, as it can reduce the morbidity and mortality associated with HSCT complications.

Moreover, we observed that the occurrence of GVHD negatively affected B-cell reconstitution, as patients who developed acute or cGVHD had lower B cells than patients who did not develop GVHD. This may be attributed to the use of immunosuppressive drugs for GVHD treatment [48, 49]. We also examined the prognostic implications of B-cell reconstitution for survival and relapse outcomes after allo-HSCT. We found that B-cell reconstitution was a protective factor for survival. This may be due to the role of B cells in preventing infections and enhancing immune tolerance after allo-HSCT [50–53]. We also found that B-cell reconstitution was associated with lower risk of relapse. This may be explained by the involvement of B cells in graft-versus-leukemia effect and antitumor immunity after allo-HSCT [54–58].

Our study demonstrated that age is a key factor influencing B-cell reconstitution and that both age and B-cell reconstitution significantly affected survival after transplantation. The older the donor age, the worse the B-cell reconstitution. For this, we conducted relative subgroup analyses (Table S11 and Fig. S7). The mechanism of the role of age factor in the process of B-cell reconstitution after transplantation remains unclear and needs further investigation. To elucidate the role of B cells in aging process, we innovatively applied mediation analysis to determine for the first time the effect of B cells as mediators on posttransplant survival. Among them, B cells at 12 months after transplantation were particularly important. We performed an in-depth analysis of bone marrow and peripheral blood B cells of patients at 12 months after transplantation, and the analysis of the developmental stages of B cells in the bone marrow revealed that the B cells of the younger patients

tended to undergo more maturation, which might suggest the mechanism by which age affects B-cell reconstitution. The analysis of peripheral blood B-cell subsets showed that CD19+ and CD20+ cells were much higher in transplanted patients than in healthy individuals, which might indicate that the immune status of the patients had not fully normalized 1 year after transplantation and might be influenced by infections and cGVHD. The mechanisms and consequences of these findings still need to be explored. Our study suggests that B cells at 12 months after transplantation are critical for assessing immune reconstitution and predicting long-term patient survival.

Van der Maas et al. [59] studied memory B-cell reconstitution in children after transplantation and mentioned that the absolute count of memory B cells in children was negatively correlated with age, and our study found that the proportion of memory B-cell was higher in older patients, because of the large standard deviation in the cohort, so there was no statistically significant difference in the final *t*-tests done with the normal group. Moreover, because of the limitation of the population of the cohort, the analysis of age-matched memory B-cell is a potential direction for future research. Furthermore, our study focused on the proportion of B cells and the proportion of B-cell subpopulations, but there is no doubt that the absolute number and proportion of posttransplantation B cells are negatively correlated with age, but figuring out how the proportion of B-cell subpopulations varies in posttransplantation patients may require more rigorous prospective studies to be realized.

The strengths of our study include the following. First, we innovatively used bone marrow samples to examine the recovery of B cells therein and illustrate the important role of B cells in bone marrow in immunity. Recent studies have shown that B cells in the bone marrow are also crucial in that they not only perform key functions in hematopoiesis but also are also an important lymphoid organ [60, 61]. Pabst summarized that CD19+ B cells are more abundant in the bone marrow than in the peripheral blood, which is in-line with our findings [62]. Second, we performed a comprehensive analysis of the time points and subgroups of the various factors and provided insights into the effects of the various factors on the immune reconstitution of the B cells during transplantation impact. More importantly, we also used PSM to eliminate confounding factors introduced by regression studies and revealed for the first time the relationship between sex and B-cell reconstitution. Moreover, we disclosed for the first time the relationship between the age-B cells-survival axes and assessed for the first time the important role of B cells as mediators in the mechanism of aging-related death. Finally, our innovative analysis of B-cell developmental stages in bone marrow provides clues to reveal the mechanism of age in B-cell reconstitution and reflects the humoral immunity of patients 1 year after transplantation by comparing the subpopulations of normal and transplanted patients' B cells in peripheral blood.

Our study has several clinical implications for the management of B-cell reconstitution and humoral immunity after allo-HSCT. First, we suggest using B-cell counts in peripheral blood as a reliable surrogate marker for B-cell reconstitution in the bone marrow, which may reduce the need for invasive bone marrow biopsies. Monitoring B-cell counts at 1, 2, 3, 6, 12, and 24 months after transplantation is crucial. Second, we identified several factors that can delay or impair B-cell reconstitution, such as sex, age, donor age, GVHD, and infections. When designing individualized treatment plans and monitoring strategies for patients undergoing allo-HSCT, it is important to consider the factors outlined above. Third, our findings indicate that B-cell reconstitution is linked to survival outcomes, especially in older patients. Therefore, improving B-cell recovery may enhance the prognosis of patients after allo-HSCT. We recommend enhancing posttransplant immune surveillance in patients over 50 years of age. Finally, this study presents new insights into the development stages and subsets of B cells in the bone marrow and peripheral blood after allo-HSCT. These findings may aid in understanding the mechanisms of B-cell maturation and diversification, as well as the interactions between B cells and other immune cells.

Our study also has some limitations. First, it is acknowledged that the use of corticosteroids can significantly impact B-cell reconstitution [63, 64]. Although corticosteroids are not commonly used in our transplant system, they are frequently used to control acute and cGVHD. However, we did not investigate the specific relationship between corticosteroids and GVHD and B-cell reconstitution, which would require the design of a specific analysis protocol that includes corticosteroid

dosage, duration, and degree of GVHD, which could be a follow-up study. Second, B-cell function was not investigated because the infusion of immunoglobulin in our transplantation system affects the observations, and future controlled observational studies could be designed to investigate function in B-cell reconstitution as this may guide the clinical management of immunoglobulin replacement. In addition, we calculated mean values and confidence intervals for B-cell counts and dynamics in different patient subgroups, which may not accurately reflect the individual variability and heterogeneity of B-cell recovery and function after HSCT. Therefore, our findings may have limited applicability to individual patients and their therapeutic strategies. Prospective studies targeting specific influences are needed to determine the applicability of B-cell reconstitution influences and prognosis to patients. Furthermore, as we are focusing on B-cell reconstitution, the reconstitution of other immune cells was not within the scope of our study, yet the role of other immune cells, including T- and NK cells, should not be overlooked. Understanding the mechanisms and dynamics of immune cell reconstitution after HSCT is crucial for comprehending the relationship between immune reconstitution and clinical outcomes. Finally, the complexity of the allo-HSCT procedure and the immune reconstitution process, which can take up to 1–2 years with many influencing factors, require intensive follow-up and observation after transplantation, making clinical studies of immune reconstitution challenging, even if prospective.

In conclusion, our study provides valuable insights into the factors that influence B-cell immune reconstitution after allo-HSCT and their prognostic impact on survival and relapse outcomes. Our findings suggest that optimizing the management of B-cell reconstitution may improve the efficacy and safety of allo-HSCT.

## Author contributions

*Conceptualization; data curation (lead); methodology; formal analysis; visualization (lead); writing—original draft; writing—review and editing*: Guangyu Zhou and Qian Zhan. *Conceptualization; writing—review and editing; supervision; project administration; funding acquisition*: Li Wang. *Data curation; methodology*: Xi Dou. *Data curation; methodology*: Lingle Huang. *Investigation*: Lin Xiang, Sicen Wu, Hongbin Zhang, Guilin Ren, Yu Zhu, Caixia Pei, Yuhong Qi, Qin Luo, Yulian Dai and Lanxiang Liu. *Supervision*: Lin Liu and Guosheng Ren. *Resources*: Jianbin Chen, Xiaoqiong Tang, Hongbin Zhang, Xin Wang, Xiaohua Luo, Zesong Yang, Xinyu Yan.

## Conflict of interest statement

The authors declare no conflicts of interest.

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## Data availability statement

For original data, please contact liwang@hospital.cqmu.edu.cn.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig S1** Bone marrow flow cytometry analysis.

**Fig S2** Peripheral blood flow cytometric analysis.

**Fig S3** Propensity Matching Score by Gender Grouping.

**Fig S4** Forest plot and sensitivity analysis of mediation effect.

**Fig S5** The Kaplan-Meier curves of myeloid and lymphoid malignancies.

**Fig S6** The boxplot of B cell percentage difference between CsA and FK506 groups.

**Fig S7** The Kaplan-Meier curves of the cohort grouped by donor age.

 

**Table S1**Information on age and sex of patients and healthy controls was monitored for B cell subsets of peripheral blood.

**Table S2** Conditioning regimens selection.

**Table S3** p-value of Nonparametric tests for factors influencing B-cell after HSCT

**Table S4** Correlation analysis for factors influencing B-cell after HSCT

**Table S5** Cutoff points for death and relapse

**Table S6** Data by gender grouping were compared.

**Table S7** Mediation effects analysis of age, B cells and survival on several timepoints after HSCT.

**Table S8** The basic information of myeloid and lymphoid malignancies groups.

**Table S9** The median value of continuous variables of myeloid and lymphoidmalignancies groups.

**Table S10** The basic information of CsA and FK506 groups.

**Table S11** The basic information of the cohort grouped by donor age.