Monocyte subsets in bone marrow grafts may contribute to a low incidence of acute graft-vs-host disease for young donors

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Abstract
Young donors are associated with a lower cumulative incidence of acute graft-vs-host disease (aGVHD) after allogeneic haematopoietic stem cell transplantation (allo-HSCT) than old donors. Although grafts are harvested from healthy donors, it is unclear whether donor age is associated with aGVHD occurrence owing to its effect on cell compositions in grafts. Moreover, the differences in monocyte subsets in grafts between young and old donors and the association between monocyte subsets in bone marrow (BM) grafts and aGVHD remain to be elucidated. In the current study, non-classical monocytes and the CD4+/CD8+ T cell ratio were remarkably decreased in BM grafts in donors <30 years old. Multivariate analysis further revealed that the level of non-classical monocytes in BM grafts (≥0.31 × 10^6/kg) was an independent risk factor for the occurrence of II-IV aGVHD. In summary, our data indicate that non-classical monocytes in BM grafts may help identify patients at high risk for aGVHD after allo-HSCT. Although further validation is required, our results suggest that the low level of non-classical monocytes and a low ratio of CD4+/CD8+ T cell in BM grafts may be correlated with the lower incidence of aGVHD in young donors.

KEYWORDS
acute graft-vs-host disease, allogeneic haematopoietic stem cell transplantation, donor graft, monocytes
1 | INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) provides a potential curative therapy for patients with haematological diseases. However, acute graft-vs-host disease (aGVHD) remains a major complication after allo-HSCT.\(^1\)\(^-\)\(^3\) The consensus for donor selection suggests that young donors are a better choice for patients, as they are associated with a lower incidence of aGVHD after allo-HSCT than old donors.\(^4\)\(^-\)\(^6\) Several studies in HLA-matched transplants have shown a lower incidence of aGVHD using grafts from young donors.\(^7\)\(^-\)\(^8\) The impact of donor age has been confirmed in the setting of haploidentical stem cell transplantation (haplo-SCT).\(^6\) Wang et al reported a lower incidence of aGVHD associated with young donors (<30 years old) in haplo-SCT based on immune tolerance induced by granulocyte colony-stimulating factor (G-CSF) and antithymocyte globulin (ATG).\(^9\) González-Vicent et al demonstrated a lower incidence of aGVHD after T cell-depleted haplo-SCT when using grafts from younger donors (<40 years old).\(^10\) Nevertheless, the underlying reason why young donors are associated with a lower incidence of aGVHD is still unknown.

The pathogenesis of aGVHD is commonly believed to be caused by exaggerated and undesirable immune responses in which there is a complex interplay between the donor cells and recipient cells. It has been reported that the different cell compositions in donor grafts are involved in the pathogenesis of aGVHD.\(^11\)\(^-\)\(^14\) The increased ratio of CD4\(^+\)/CD8\(^+\) T cells in donor bone marrow (BM) grafts is often utilized as a biomarker for a high incidence of aGVHD. Moreover, our recent study reported that an imbalance in macrophage polarization in donor BM grafts, characterized by a high M1/M2 macrophage ratio, exhibited a high incidence of aGVHD.\(^15\) These studies suggest that the cell compositions in donor grafts may help to identify patients who are at high risk for aGVHD.

Given that grafts are harvested from healthy donors, donor age has been reported to be associated with the cell compositions in donor grafts. Yakoub-Agha et al reported that CD8\(^-\)-naive T cells in grafts are negatively associated with donor age, whereas the ratio of CD4\(^+\)/CD8\(^+\) T cells and CD8\(^+\) effector memory T cells in grafts are positively associated with donor age.\(^16\) Furthermore, a high percentage of CD14\(^+\) monocytes was reported in grafts of young donors.\(^17\) In humans, circulating monocytes are classified into three subsets: classical, intermediate and non-classical monocytes.\(^18\)\(^-\)\(^19\) Classical monocytes are highly phagocytic and are important scavenger cells. Intermediate monocytes have antigen presentation and angiogenesis functions. Non-classical monocytes demonstrate proinflammatory behaviour and secrete inflammatory cytokines in response to infection. In this regard, the imbalance in monocyte subsets has been reported to play a critical role in the occurrence and development of many inflammatory disorders. These findings suggest that the imbalance in monocyte subsets is a promising predictor for risk stratification in inflammatory diseases.\(^20\)\(^-\)\(^26\) However, the differences in monocyte subsets between young and old donors and the association between monocyte subsets in BM grafts and aGVHD remain to be elucidated.

Therefore, the current study was performed to determine whether donor age is associated with aGVHD occurrence owing to its effect on cell compositions in BM grafts. Our aim was to provide new insights into why young donors are a better choice for patients undergoing allo-HSCT than old donors.

2 | MATERIALS AND METHODS

2.1 | Patients and their healthy donors

A total of 83 patients who underwent allo-HSCT and their own healthy donors were enrolled at Peking University People’s Hospital. The donor cohort comprised 59 males and 24 females, aged 16–63 years old (median, 39 years old). As shown in Table 1, the enrolled donors were designated into young (age < 30 years), middle-aged (30 years ≤ age≤45 years) and old (age > 45 years) donor groups. Blood cell counts including white blood cell (WBC), neutrophils, lymphocytes and monocytes in peripheral blood (PB) of healthy donors are analysed at three-time points: before G-CSF mobilization, before G-CSF-mobilized BM (G-BM, on the fourth day after G-CSF mobilization) harvesting and before G-CSF-mobilized peripheral blood (G-PB, on the fifth day after G-CSF mobilization) apheresis. Most of the characteristics including the underlying diseases of their related patients showed no significant differences among the three donor age groups, whereas the lymphocyte counts were significantly lower in middle-aged donor group (Table 1). Subsequently, the effect of the monocyte subsets in BM grafts on the occurrence of aGVHD was evaluated.

The current study was approved by the Ethics Committee of Peking University People’s Hospital, and written informed consent was obtained from all patients and donors in compliance with the Declaration of Helsinki.

2.2 | Transplantation protocols

Donor selection, conditioning therapy, graft harvesting and the prevention of GVHD have been described previously.\(^27\)\(^-\)\(^29\) Donors were ranked based on the best HLA match, age (younger preferred) and donor-recipient sex (same preferred). Donors were injected subcutaneously with G-CSF at 5 µg/kg daily for five consecutive days. For haplo-SCT, recipients were treated with a modified busulfan/cyclophosphamide plus ATG regimen before the infusion of unmanipulated G-BM and G-PB. GVHD prophylaxis was performed with cyclosporine, mycophenolate mofetil and short-course methotrexate. All transplantation recipients received cyclosporine A (CsA), mycophenolate mofetil (MMF) and short-term methotrexate (MTX) as GVHD prophylaxis. The dosage...
of CsA was 2.5 mg/kg/d IV from day 9 until bowel function returned to normal, at which point, patients were switched to oral CsA. Every 12 hours, 0.5 g of MMF was administered orally from day 9 and was discontinued after engraftment. MTX was administered intravenously at 15 mg/m$^2$ on day 1 and then at 10 mg/m$^2$ on days 3, 5 and 11 in haplo-SCT.

### TABLE 1  Characteristics of donors and their related patients

| Characteristics | Young donor (n = 24) | Middle-aged donor (n = 35) | Old donor (n = 24) | $P^*$ | $P^{**}$ | $P^{***}$ |
|-----------------|----------------------|---------------------------|-------------------|-------|---------|---------|
| Gender, male/female | 15/9                | 25/10                      | 19/5              | .47   | .20     | .50     |
| Weight (kg)$^a$ | 67.5 (47-95)        | 71 (47-90)                 | 69 (45-100)       | .33   | .82     | .33     |
| BMI (kg/m$^2$)$^a$ | 22.81 (18.91-30.49) | 24.49 (19.83-29.88)        | 23.17 (16.94-33.41) | .27   | .61     | .66     |
| Blood cell counts (before G-CSF mobilization) | | | | | |
| WBC ($\times 10^9$/L)$^a$ | 6.54 (3.86-12.66) | 5.93 (3.89-9.95) | 5.86 (3.54-9.28) | .12   | .18     | .96     |
| Neutrophils ($\times 10^9$/L)$^a$ | 3.15 (1.97-7.80) | 3.28 (1.51-7.67) | 3.40 (1.64-5.27) | .42   | .34     | .83     |
| Lymphocytes ($\times 10^9$/L)$^a$ | 2.30 (1.22-3.95) | 1.89 (0.92-3.60) | 2.08 (1.39-3.30) | .02   | .09     | .45     |
| Monocytes ($\times 10^9$/L)$^a$ | 0.44 (0.17-0.86) | 0.45 (0.21-0.60) | 0.44 (0.17-0.74) | .43   | .84     | .55     |
| Blood cell counts (before G-BM harvesting) | | | | | |
| WBC ($\times 10^9$/L)$^a$ | 33.05 (14.90-47.50) | 31.29 (15.60-44.90) | 31.03 (21.87-45.00) | .26   | .80     | .38     |
| Neutrophils ($\times 10^9$/L)$^a$ | 27.20 (12.60-40.70) | 26.63 (13.30-38.90) | 27.34 (18.00-38.20) | .27   | .89     | .32     |
| Lymphocytes ($\times 10^9$/L)$^a$ | 3.05 (1.38-4.90) | 2.70 (1.50-5.29) | 2.67 (0.87-4.40) | .21   | .24     | .90     |
| Monocytes ($\times 10^9$/L)$^a$ | 1.59 (0.90-4.20) | 3.44 (1.51-7.80) | 1.68 (0.81-3.00) | .16   | .95     | .14     |
| Blood cell counts (before G-PB harvesting) | | | | | |
| WBC ($\times 10^9$/L)$^a$ | 42.00 (31.27-60.30) | 41.70 (22.03-57.47) | 38.00 (28.40-58.30) | .41   | .21     | .66     |
| Neutrophils ($\times 10^9$/L)$^a$ | 36.4 (27.15-54.10) | 37.10 (27.15-54.10) | 33.45 (24.5-50.9) | .43   | .21     | .65     |
| Lymphocytes ($\times 10^9$/L)$^a$ | 3.10 (2.20-4.60) | 3.00 (1.30-5.50) | 3.17 (1.66-6.93) | .50   | .56     | .26     |
| Monocytes ($\times 10^9$/L)$^a$ | 2.10 (1.00-4.00) | 2.10 (0.90-4.02) | 1.81 (1.00-3.40) | .53   | .14     | .30     |

The underlying diseases of their related patients

| AML | 13 | 25 | 17 | .17 | .23 | .96 |
| ALL | 10 | 9  | 5  | .20 | .12 | .67 |
| MDS | 1  | 1  | 2  | .78 | .55 | .35 |

Abbreviations: G-BM, G-CSF-primed bone marrow; G-CSF, granulocyte colony-stimulating factor; G-PB, G-CSF-primed peripheral blood; WBC, white blood cell.

$^a$ Data are reported as median (range).

$^*P$-value between young and middle-aged donors.

$^{**}P$-value between young and old donors.

$^{***}P$-value between middle-aged and old donors.

Overall survival (OS) was defined as the time from transplantation to death from any cause.

### 2.4  Identification and analysis of cell compositions in donor grafts

Samples from G-BM grafts were labelled with the following monoclonal antibodies and appropriate isotypes: CD45-PerCP, CD3-APC, CD4-PE and CD8-FITC (BioLegend). The immunophenotype of cell compositions in donor grafts was quantified via flow cytometry. The percentages of CD3$^+$ T cells, CD3$^+$CD4$^+$ T cells and CD3$^+$CD8$^+$ T cells are expressed as a fraction of low side scatter and CD45$^+$ lymphocyte gate. The absolute numbers of graft compositions were calculated as the percentages of these cells multiplied by the percentages of lymphocytes multiplied by the total nucleated cell and divided by the actual patient weight to calculate the numbers of cells per kilogram.

### 2.3  Clinical definitions and assessments

aGVHD was diagnosed and graded based on clinical symptoms and/or skin, oral mucosa, liver or gut biopsy, and disease severity was scored using published consensus criteria.$^{30-32}$ Relapse was defined by morphologic evidence of disease in PB, BM, or extramedullary sites or by the recurrence and sustained presence of pre-transplantation chromosomal abnormalities. Disease-free survival (DFS) was defined as the probability of being alive and free of disease at any point in time, with death or disease relapse considered events.

Overall survival (OS) was defined as the time from transplantation to death from any cause.
2.5 | Characterization of monocyte subsets

As previously described, classical monocytes, intermediate monocytes and non-classical monocytes were identified as CD14<sup>high</sup>CD16<sup>−</sup>, CD14<sup>−</sup>CD16<sup>+</sup> and CD14<sup>−</sup>CD16<sup>high</sup>, respectively. The relative frequencies of these monocyte subsets are expressed as a fraction of the CD14<sup>+</sup> monocyte subset. Samples from G-BM grafts were labelled with CD14 and CD16 for monocyte subset analyses. Multiparameter flow cytometric analyses were performed using a BD LSFRFortessa cell analyser (BD Biosciences). The data were analysed using BD LSFRFortessa software (BD Biosciences). The absolute numbers of monocyte subsets in BM grafts were calculated as the percentages of these cells multiplied by the percentages of total CD14<sup>+</sup> cells multiplied by the total nucleated cell and divided by the actual patient weight to calculate the numbers of cells per kilogram.

2.6 | Statistical analysis

Patient variables were compared using the chi-square test for categorical variables. A Mann-Whitney U test was performed to analyse continuous variables. Cumulative incidences of aGVHD and relapse were estimated to accommodate competing risks. Death from any cause was defined as a competing risk for aGVHD and relapse. Comparisons between cumulative incidences were performed by the Gray test. The probabilities of OS and DFS were estimated with the Kaplan-Meier method and compared using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model for survival to identify the independent prognostic variables. The parameters with $P < .1$ according to the univariate analysis were entered into a multivariate model. To analyse the association between donor characteristics and graft cell composition, logistic regression analyses were conducted to determine the independent donor factors involved in donor dichotomous variables selected from the univariate analysis. Analyses were performed using GraphPad Prism 6.0 and SPSS (IBM Corporation) version 19 software, and the R software package (version 2.6.1; http://www.r-project.org) was used for competing risk analysis. $P$-values < .05 were considered statistically significant.

3 | RESULTS

3.1 | The percentages and numbers of classical and non-classical monocytes in BM grafts were different among young, middle-aged and old donors

The representative gating strategy for classical, intermediate and non-classical monocytes in BM grafts is shown in Figure 1A. The enrolled donors were designated into young (age < 30 years), middle-aged (30 years ≤ age ≤ 45 years) and old (age > 45 years) groups. Compared with young group, the percentages of classical monocytes in BM grafts (Figure 1B; 58.68%±2.83% vs 68.19%±1.86%; $P = .007$) were significantly lower in old group, whereas the percentages of non-classical monocytes (Figure 1D; 18.88%±1.32% vs 14.68%±1.28%; $P = .03$) were significantly higher in old group.

Moreover, the numbers of classical monocytes (Figure 1E; 2.00 ± 0.28 vs 1.12 ± 0.09; $P = .005$), intermediate monocytes (Figure 1F; 0.17 ± 0.02 vs 0.07 ± 0.01; $P < .0001$) and non-classical monocytes (Figure 1G; 0.67 ± 0.11 vs 0.23 ± 0.02; $P = .0004$) in BM grafts were significantly higher in old group than those in young group.

3.2 | Different immune cell subsets in BM grafts among donors of different ages

The number of lymphocytes (Figure 2B; 2.47 ± 0.15 vs 2.98 ± 0.18; $P = .03$) and CD3<sup>+</sup> T cells (Figure 2C; 1.55 ± 0.10 vs 1.89 ± 0.11; $P = .03$) in BM grafts were significantly lower in old group than in young group. Moreover, the number of CD8<sup>+</sup> T cells in BM grafts was significantly lower in middle-aged group (Figure 2E; 0.54 ± 0.04 vs 0.68 ± 0.04; $P = .04$) and old group (Figure 2E; 0.40 ± 0.04 vs 0.68 ± 0.04; $P < .0001$) than in young group. Old donors had a lower number of CD8<sup>+</sup> T cells in BM grafts (Figure 2E; 0.40 ± 0.04 vs 0.54 ± 0.04; $P = .02$) than middle-aged donors. The numbers of total nucleated cells (Figure 2A) and CD4<sup>+</sup> T cells (Figure 2D) showed no significant differences among the three age groups. The ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T cells was significantly higher in middle-aged group (Figure 2F; 2.11 ± 0.14 vs 1.63 ± 0.12; $P = .02$) and old group (Figure 2F; 3.40 ± 0.44 vs 1.63 ± 0.12; $P = .0003$) than in young group. Therefore, the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T cells in BM grafts was highest in old donor group among the three age groups.

3.3 | WBC counts before G-CSF mobilization predicted the percentages of classical and non-classical monocytes in BM grafts

Positive correlations were demonstrated between WBC counts before G-CSF mobilization and the percentage of classical monocytes (Figure 2G; $r = .23$ (95% confidence interval (CI), 0.02, 0.43); $P = .03$). However, inverse correlations were found between WBC counts before G-CSF mobilization and the percentage of non-classical monocytes (Figure 2H; $r = -.24$ (−0.43, −0.02); $P = .03$). In addition, positive correlations were demonstrated between lymphocyte counts before G-CSF mobilization (Figure 2I; $r = .24$ (0.02, 0.43); $P = .03$), monocyte counts before G-CSF mobilization (Figure 2J; $r = .28$ (0.07, 0.47); $P = .01$) and the ratio of classical/non-classical monocytes.

3.4 | Donor age was independently correlated with monocyte subsets and CD4<sup>+</sup>/CD8<sup>+</sup> T cells in BM grafts

To clarify the relationship between donor characteristics and monocyte subsets, the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T cells in BM grafts, donor age, sex, weight, WBC counts, neutrophils, lymphocytes and monocytes was analysed with univariate and multivariate analyses.
As shown in Table 2, multivariate analysis revealed that donor age ≥ 30 years was associated with high numbers of classical monocyte (2.72, 1.01-7.35, \(P = .04\)), intermediate monocyte (9.05, 2.73-30.00, \(P < .0001\)) and non-classical monocytes (7.40, 2.14-25.57, \(P = .002\)) in BM grafts. Moreover, donor age ≥ 30 years (6.39, 2.09-19.54, \(P = .001\)) was associated with a high ratio of CD4⁺/CD8⁺ T cells in BM grafts.

### 3.5 Percentages and numbers of classical, intermediate and non-classical monocytes in BM grafts of grade II-IV aGVHD patients

As shown in Table 3, most of the demographic and clinical characteristics showed no significant differences between patients with grade 0-I aGVHD and those with grade II-IV aGVHD.
As illustrated in Figure 3, when compared with grade 0-I aGVHD patients, the percentage of classical monocytes (Figure 3A; 58.15%±3.16% vs 65.61%±1.16%; \( P = .04 \)) was significantly lower in grade II-IV aGVHD patients, whereas the percentage of non-classical monocytes (Figure 3C; 20.85%±1.47% vs 15.54%±0.72%; \( P = .001 \)) was significantly higher in grade II-IV aGVHD patients. Moreover, the number of non-classical monocytes (Figure 3F; 0.60 ± 0.11 vs 0.34 ± 0.03; \( P = .003 \)) was significantly higher in grade II-IV aGVHD patients than those with grade 0-I aGVHD patients.

### 3.6 Percentages and numbers of classical, intermediate and non-classical monocytes in BM grafts affect the severity of aGVHD

To evaluate whether the severity of aGVHD is associated with the level of monocytes in BM grafts, the percentages and numbers of classical monocytes, intermediate monocytes and non-classical monocytes were compared between patients with grade III-IV aGVHD and those with grade I-II aGVHD. The percentage of classical monocytes was significantly lower in grade III-IV aGVHD patients than in grade I-II aGVHD patients (Figure 3G; 54.53%±6.18% vs 64.73%±1.56%; \( P = .02 \)) and non-aGVHD patients (Figure 3G; 54.53%±6.18% vs 64.21%±1.61%; \( P = .04 \)), whereas the percentage of non-classical monocytes (Figure 3I; 21.05%±2.72% vs 15.80%±1.08%; \( P = .04 \)) was significantly higher in grade III-IV aGVHD patients than those with non-aGVHD patients. Moreover, the number of non-classical monocytes (Figure 3J; 0.57 ± 0.15 vs 0.31 ± 0.04; \( P = .02 \)) was significantly higher in grade III-IV aGVHD patients than those with non-aGVHD patients.

### 3.7 The monocyte subsets in BM grafts were associated with the incidence of grade II-IV aGVHD but did not have a significant influence on relapse or survival

The enrolled patients were designated into the high BM graft group or the low BM graft group according to the median numbers of the transplanted classical monocytes (1.22 × 10⁶/kg), intermediate monocytes (0.10 × 10⁶/kg) or non-classical monocytes (0.31 × 10⁶/kg) in BM grafts.

The cumulative incidence of grade II-IV aGVHD in low non-classical monocyte group was significantly lower than that in high non-classical monocyte group (Figure 4C; 19.5% (9.4%-35.4%) vs 42.9% (28.1%-58.9%), \( P = .04 \)). After a median follow-up of 764 days (range 49-989 days), the cumulative incidence of relapse (Figure 4D-F) and the probabilities of DFS and OS (Figure S1) showed no significant differences between the different monocyte subsets groups.

### 3.8 Non-classical monocytes in BM grafts were an independent risk factor for the occurrence of grade II-IV aGVHD

As shown in Table 4, the association between donor characteristics and the occurrence of grade II-IV aGVHD was analysed with a univariate analysis. The percentage of classical monocytes in BM grafts was negatively correlated with the incidence of grade II-IV aGVHD. However, the percentage of non-classical monocytes in BM grafts was positively correlated with the incidence of grade II-IV aGVHD. Multivariate analysis demonstrated that non-classical monocytes in BM grafts, which accounted for \( 0.31 \times 10^6 \)kg (2.32, 1.01-5.33, \( P = .04 \)), was independently correlated with a high incidence of grade II-IV aGVHD after allo-HSCT.
In the current study, we found that classical monocytes were significantly increased in donors <30 years old, whereas non-classical monocytes and the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T cells were remarkably decreased in BM grafts in donors <30 years old. In addition, patients who received a BM graft with a high proportion of non-classical monocytes exhibited a significantly high incidence of aGVHD, whereas the percentage of classical monocytes in BM grafts was negatively correlated with the incidence of aGVHD. Multivariate...
analysis further demonstrated that non-classical monocytes in BM grafts (≥0.31 × 10^6/kg) were independently correlated with a high incidence of grade II-IV aGVHD after allo-HSCT.

Previous work revealed that young donors are correlated with a low incidence of aGVHD. However, the underlying reason why young donors are associated with a lower incidence of aGVHD than old donors remains to be clarified. Several cell compositions in grafts have been reported to be useful for identifying patients at high risk for aGVHD. Luo et al found that a CD4^+ /CD8^+ T cell ratio ≥1.16 in BM grafts was associated with a high risk for aGVHD. Subsequently, in a controlled, open-label, randomized trial, prophylaxis with a low-dose corticosteroid for high-risk patients who were infused with BM grafts at a CD4^+ /CD8^+ T cell ratio of ≥1.16 significantly decreased the incidence and delayed the onset of aGVHD. Moreover, our previous study found that young donors were associated with a higher number of CD14^+ monocytes in donor grafts. The current study provides new evidence that BM grafts harvested from young donors contain low percentages of non-classical monocytes but high percentages of classical monocytes. Moreover, BM grafts harvested from young donors contain a lower ratio of CD4^+ /CD8^+ T cells in grafts compared to older donors. These observations suggest that non-classical monocytes may play an important role in the occurrence of aGVHD by inducing TNF-α production and promoting the induction of proinflammatory cells. Therefore, further functional studies are needed to elucidate the underlying mechanism of non-classical monocytes in BM grafts affecting the occurrence of aGVHD.

However, we are aware that further studies are needed to clarify the effect of monocyte subsets in PB grafts on aGVHD. Moreover, further clarification is required to determine whether...
In summary, the current study shows that donor age is positively correlated with the percentage of non-classical monocytes and the ratio of CD4+/CD8+ T cells, whereas negatively correlated with the percentage of classical monocytes in BM grafts. Moreover, our data indicate that non-classical monocytes in BM grafts may help to identify patients who are at high risk for aGVHD after allo-HSCT. Although further validation is required, our results suggest that the low level of non-classical monocytes and a low ratio of CD4+/CD8+ T cell in BM grafts may be correlated with the lower incidence of aGVHD in young donors.

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**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**AUTHOR CONTRIBUTIONS**

Qi Wen: Formal analysis (equal); Investigation (lead); Methodology (lead); Project administration (lead); Validation (equal); Writing-original draft (lead); Writing-review & editing (lead). Hongyan Zhao: Formal analysis (equal); Investigation (equal); Project administration (equal); Validation (equal); Writing-original draft (equal); Writing-review & editing (equal). Wei-Li Yao: Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Validation (equal); Writing-original draft (equal); Writing-review & editing (supporting). Yuanyuan Zhang: Formal analysis (equal); Investigation (equal); Project administration (equal); Validation (equal). Hai-Xia Fu: Formal analysis (equal); Investigation (equal); Project administration (equal); Supervision (supporting). Yu Wang: Formal analysis (supporting); Investigation (supporting); Project administration (supporting); Supervision (supporting). Lan-Ping Xu: Formal analysis (supporting); Investigation (supporting); Project administration (supporting); Supervision (supporting). XiaoHui Zhang: Formal analysis (supporting); Investigation (supporting); Project administration (supporting); Supervision (supporting). Yuan Kong: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (equal); Project administration (lead); Supervision (lead); Validation (lead); Writing-original draft (lead); Writing-review & editing (supporting).
REFERENCES

1. Ferrara JLM, Levine JE, Reddy P, et al. Graft-versus-host disease. Lancet. 2009;373(9672):1551-61.

2. Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. N Engl J Med. 2017;377(22):2167-2179.

3. Kopolovic I, Ostro J, Tsubota H, et al. A systematic review of transfusion-associated graft-versus-host disease. Blood. 2015;126(3):406-414.

4. Ciurea SO, Al Malki MM, Kongtim P, et al. The European Society for Blood and Marrow Transplantation (EBMT) consensus recommendations for donor selection in haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. 2020;55(11):12-24.

5. Little A-M, Green A, Harvey J, et al. BSHI Guideline: HLA matching and donor selection for hematopoietic progenitor cell transplantation. Int J Immunogenet. 2016;43(5):263-286.

6. McCurdy SR, Zhang M-J, St. Martin A, et al. Effect of donor characteristics on the immune cell composition of mixture allografts of granulocyte-colony-stimulating factor-mobilized marrow harvests and peripheral blood. Transfusion. 2015;55(12):2874-2881.

7. Luo X-H, Chang Y-J, Xu L-P, et al. The impact of graft composition on clinical outcomes in unmanipulated HLA-mismatched/haploidentical hematopoietic stem cell transplantation. Bone Marrow Transplant. 2019;54(9):1419-1433.

8. Gaziev J, Isgrò A, Marziali M, et al. Higher CD34(+) and CD34(+) cell doses in the graft increase the incidence of acute GVHD in children receiving BMT for thalassemia. Bone Marrow Transplant. 2012;47(1):107-114.

9. Impola U, Larjo A, Salmenniemi U, et al. Graft immune cell composition associates with clinical outcome of allogeneic hematopoietic stem cell transplantation in patients with AML. Front Immunol. 2016;7:523.

10. Patel SS, Rybicki LA, Corrigan D, et al. Effect of bone marrow CD34+ cells and T-cell subsets on clinical outcomes after myeloablative allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2018;54(5):775-781.

11. Wen Q, Kong Y, Zhao H-Y, et al. G-CSF-induced macrophage polarization and mobilization may prevent acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2019;54(9):1419-1433.

12. Yakoub-Agha I, Saule P, Depil S, et al. Comparative analysis of naïve and memory CD4(+) and CD8(+) T-cell subsets in bone marrow and G-CSF–mobilized peripheral blood stem cell allografts: impact of donor characteristics. Exp Hematol. 2007;35(6):861-871.

13. Wang Y-T, Zhao X-Y, Zhao X-S, et al. The impact of donor characteristics on the immune cell composition of mixture allografts of granulocyte-colony-stimulating factor-mobilized marrow harvests and peripheral blood. Transfusion. 2015;55(12):2874-2881.

14. Auffray C, Sieweke MH, Geissmann F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu Rev Immunol. 2009;27(1):669-692.

15. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Monocyte subtypes in blood. Blood. 2010;116(16):e74-e80.

16. Heine GH, Ortiz A, Massy ZA, et al. Monocyte subpopulations and cardiovascular risk in chronic kidney disease. Nat Rev Nephrol. 2012;8(6):362-369.

17. Reinhardt-Heller K, Hirschberg I, Vogl T, et al. Characterization of monocyte subtypes in recipients after transplantation of bone marrow harvests and peripheral blood. Transpl Immunol. 2018;45:48-54.

18. Lo S-C, Lee W-J, Chen C-Y, et al. Intermediate CD14++CD16+ monocyte predicts severe coronary stenosis and extensive plaque involvement in asymptomatic individuals. Int J Cardiovasc Imaging. 2017;33(8):1223-1236.

19. Reinhardt-Heller K, Hirschberg I, Lang P, et al. Increase of intermediate monocytes in graft-versus-host disease: correlation with MDR1(+)Th17.1 levels and the effect of prednisolone and 1alpha, 25-Dihydroxyvitamin D3. Biol Blood Marrow Transplant. 2017;23(12):2057-2064.

20. Randolph GJ, Jakubzick C, Qu C. Antigen presentation by monocytes and monocyte-derived cells. Curr Opin Immunol. 2008;20(1):52-60.

21. Murray PJ. Immune regulation by monocytes. Semin Immunol. 2018;35:12-18.

22. Gordon S. Targeting a monocyte subset to reduce inflammation. Circ Res. 2012;110(12):1546-1548.

23. Huang X-J, Zhu H-H, Chang Y-J, et al. The superiority of haploidentical related stem cell transplantation over chemotherapy alone as postremission treatment for patients with intermediate- or high-risk acute myeloid leukemia in first complete remission. Blood. 2012;119(23):5584-5590.

24. Wang Y, Chen H, Chen J, et al. The consensus on the monitoring, treatment, and prevention of leukemia relapse after allogeneic hematopoietic stem cell transplantation in China. Cancer Lett. 2018;438:63-75.

25. Xu L, Chen HU, Chen J, et al. The consensus on indications, conditioning regimen, and donor selection of allogeneic hematopoietic cell transplantation for hematological diseases in China—recommendations from the Chinese Society of Hematology. J Hematol Oncol. 2018;11(1):33.

26. Cahn JY, Klein JP, Lee SJ, et al. Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Societe Francoise de Greffe de Moelle et Therapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. Blood. 2005;106:1495-1500.

27. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997;97:855-864.
32. Penack O, Marchetti M, Ruutu T, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol*. 2020;7:e157-e167.
33. Fadini GP, de Kreutzenberg SV, Boscaro E, et al. An unbalanced monocyte polarisation in peripheral blood and bone marrow of patients with type 2 diabetes has an impact on microangiopathy. *Diabetologia*. 2013;56(8):1856-1866.
34. Zhao H-Y, Lyu Z-S, Duan C-W, et al. An unbalanced monocyte macrophage polarization in the bone marrow microenvironment of patients with poor graft function after allogeneic haematopoietic stem cell transplantation. *Br J Haematol*. 2018;182(5):679-692.
35. Chang Y-J, Xu L-P, Wang YU, et al. Controlled, randomized, open-label trial of risk-stratified corticosteroid prevention of acute graft-versus-host disease after haploidentical transplantation. *J Clin Oncol*. 2016;34(16):1855-1863.
36. Choi SW, Reddy P. Current and emerging strategies for the prevention of graft-versus-host disease. *Nat Rev Clin Oncol*. 2014;11(9):536-547.
37. Levine JE, Logan BR, Wu J, et al. Acute graft-versus-host disease biomarkers measured during therapy can predict treatment outcomes: a Blood and Marrow Transplant Clinical Trials Network study. *Blood*. 2012;119(16):3854-3860.
38. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leukoc Biol*. 2007;81(3):584-592.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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