Impact of allogeneic hematopoietic stem cell transplantation on pediatric acute myeloid leukemia of FAB subtypes M4 and M5

Yu-juan Xue
Peking University People's Hospital

Pan Suo
Peking University People's Hospital

Yi-fei Cheng
Peking University People's Hospital

Ai-dong Lu
Peking University People's Hospital

Yu Wang
Peking University People's Hospital

Ying-xi Zuo
Peking University People's Hospital

Jun Wu
Peking University People's Hospital

leping zhang (✉ zhangleping1964@126.com)
Peking University People's Hospital

Xiao-jun Huang
Peking University People's Hospital

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Abstract

**Background:** FAB-M4 and M5 are unique subgroups of pediatric acute myeloid leukemia. However, for these patients, few studies have demonstrated the clinical and biological characteristics and efficacy of hematopoietic stem cell transplantation (HSCT), and especially haplo-HSCT.

**Procedure:** We retrospectively evaluated the outcomes of 70 children with FAB-M4/M5 enrolled in our center from January 2013 to December 2017.

**Results:** Of the patients, 32, 23, and 15 were in low-risk, intermediate-risk, and high-risk groups, respectively. T(16;16), inv16/CBFB-MYH11 was the most frequent cytogenetic abnormality. Among detected genetic alterations, WT1 was mutated at the highest frequency, followed by FLT3-ITD, NPM1, and CEBPA. Thirty-three patients received HSCT (haplo-HSCT = 30), of which four, 18, and 11 were in low-risk, intermediate-risk, and high-risk groups, respectively. For all patients, the 3-year overall survival (OS), event-free survival (EFS), and cumulative incidence of relapse (CIR) were 85.3 ± 4.3%, 69.0 ± 5.7%, and 27.9 ± 5.2%, respectively. By multivariate analysis, low-risk stratification predicted superior OS, EFS, and PLT ≤ 50 × 10^9/L at diagnosis, with FLT3-ITD mutations predicting higher CIR and poorer EFS. In intermediate- and high-risk groups, HSCT was independently associated with higher EFS and lower CIR. With a median post-transplant observation time of 30.0 months, the 3-year OS, EFS, CIR, and non-relapse mortality in the haplo-HSCT group were 74.2 ± 8.6%, 68.3 ± 8.9, 24.6 ± 7.6%, and 6.6 ± 4.1%, respectively.

**Conclusions:** Risk-oriented treatment is important for pediatric FAB-M4/M5. For intermediate- and high-risk groups, HSCT significantly improved survival and haplo-HSCT might be a viable alternative approach.

Background

The French-American-British (FAB) cooperative team developed the first comprehensive morphological-histochemical classification system for acute myeloid leukemia (AML), categorizing AML into major subtypes based on the morphological and immunohistochemical detection of lineage markers [1, 2]. Acute myelomonocytic lineage leukemia, namely FAB subtypes M4 and M5, comprises approximately one-third of pediatric AML [3, 4].

As two unique AML subgroups with monocytic morphology and cytochemical features, FAB-M4 and M5 exhibit special clinical and biological heterogeneity [5–7]. Pediatric FAB-M4/M5 often presents with a high initial white blood cell count (WBC) and extramedullary infiltration at diagnosis, with a reported 5-year event-free survival (EFS) of 50–65% [4, 8]. According to studies on the cytogenetics and molecular biology of childhood AML, the core-binding factor (CBF) mutation-inversion16 (p13;1q22), t(16;16) (p13;q22), and t(8;21)(q22;q22) are common molecular genetic abnormalities in FAB-M4/M5, which are often identified as low-risk markers [4, 9]. For children with such alterations, favorable outcomes can be achieved with chemotherapy alone [10]. However, the long-term prognosis of other children with FAB-M4/M5 without the CBF mutation is not good, which is especially true for high-risk children with complex karyotypes [11], FMS-like tyrosine kinase 3 (FLT3) mutations [11, 12], or t(v;11q23)[13]. Currently, the
effect of allogeneic hematopoietic stem cell transplantation (HSCT), and especially haploidentical HSCT (haplo-HSCT), on the intermediate- and high-risk group is unclear. Few studies have addressed the clinical and biological characteristics of pediatric FAB-M4/M5 and identified the efficacy of HSCT for this group. Thus, to better characterize pediatric FAB-M4/M5 and investigate the efficacy of HSCT, and especially haplo-HSCT, for such children, we retrospectively analyzed the outcomes of children with FAB-M4/M5 at our center.

Materials And Methods

Patients

All newly diagnosed consecutive AML patients treated at the Peking University People's Hospital from January 2013 to December 2017 were enrolled in this study if the following criteria were met: (1) between 0 and 18 years of age, (2) with FAB type M4/M5, (3) complete at least two cycles of chemotherapy, and (4) be in first complete remission (CR1). FAB-M4/M5 from myelodysplastic syndrome (MDS) or chronic myelogenous leukemia (CML) were excluded. Figure 1 shows the overall profile of the enrolled patients, including risk classification and donor availability.

Diagnosis and risk classifications

The initial diagnosis of childhood AML was based on the recommendations of the International BFM Study Group AML Committee [14]. All children newly diagnosed with AML were tested for the presence of t(8;21)(q22;q22), inversion 16(p13;1q22)/t(16;16) (p13;q22), t(6;9)(p23;q34.1), t(v;11q23), t(9;22) (q34;q11), and related molecular transcripts, namely RUNX1-RUNX1T1, CBFB-MYH11, DEK-NUP214, KMT2A-rearrangements, and BCR-ABL1. Detection of other molecular abnormalities included mutations in NPM1 and WT1, internal tandem duplication of FLT3 mutation (FLT3-ITD), and biallelic mutations in CEBPA. We used chromosome karyotype analysis performed by the R-banding method, fluorescence in situ hybridization, and multiple reverse transcription polymerase chain reaction to detect cytogenetics and molecular abnormalities in all enrolled patients.

According to the cytogenetic abnormalities at diagnosis, patients were classified as low-, intermediate-, or high-risk groups [14]. Details are as follows: low-risk, t (8;21) (q22;q22)/RUNX1-RUNX1T1, inv(16) or t(16;16) (p13;q22)/CBFB-MYH11, biallelic mutations in CEBPA, NPM1 mutation without FLT3-ITD; high-risk, t(6;9)(p23;q34.1)/DEK-NUP214, t(v;11q23)/KMT2A-rearrangement (except t(1;11) (q21;q23)/MLLT11-KMT2A and t(9;11)(p12;q23)/MLLT3-KMT2A), t(9;22)(q34;q11)/BCR-ABL1, FLT3-ITD, complex karyotypes (defined as at least three chromosome abnormalities in the absence of one of the WHO-designated recurring translocations or inversions), –5/5q-, –7/7q-, abn(3q); intermediate-risk, cytogenetic abnormalities not classified as low- or high-risk.

Treatment protocols
All patients received 1–2 cycles of induction chemotherapy of I(D)AE, including idarubicin/daunorubicin, cytarabine, and etoposide. Consolidation chemotherapy regimens included HD Ara-c, HA, and I(D)AE. During the consolidation period, these three regimens were consecutively applied for 12–18 months, and then withdrawal follow-up began. A total of 4–6 rounds of HD Ara-c were administrated during the entire consolidation treatment and the cumulative dose of anthracyclines was equivalent to DNR 350 mg/m².

All patients received intrathecal chemotherapy with methotrexate (MTX), cytarabine, and dexamethasone regularly as central nervous system leukemia (CNSL) prophylaxis. Over the total treatment course, intrathecal treatment was performed 4–8 times. The detailed chemotherapy protocol is shown in (Supp. TABLE 1). When a suitable donor was available, eligible patients in intermediate- and high-risk groups could proceed to HSCT after receiving 2–4 cycles of consolidation therapy according to the wishes of their guardian and the recommendation of their attending physicians.

We used granulocyte colony-stimulating factor-mobilized bone marrow cells plus peripheral blood stem cells as a graft resource. In matched sibling transplants, the pre-conditioning treatment was a modified BU/CY regimen (busulfan-cyclophosphamide), which included the following: hydroxyurea (80 mg/kg per day, orally) on day −10; Ara-c (2 g/m² per day, intravenously) on day −9; busulfan (Bu, 3.2 mg/kg per day, intravenously) on days −8 to −6; cyclophosphamide (Cy, 1.8 g/m² per day, intravenously) on days −5 to −4; methyl-N-(2-chloroethyl)-N-cyclohexyl-N-nitrosourea (Me-CCNU, 250 mg/kg per day, orally) on day −3.

For haplo-transplants, the pre-conditioning treatment was a modified BU/CY regimen combined with anti-human thymocyte immunoglobulin (ATG), consisting of the following: Ara-c (4 g/m² per day, intravenously) on days −10 to −9; Bu, Cy, and Me-CCNU (same as previous described); ATG (2.5 mg/kg per day, intravenously) on days −5 to −2. The stem cells for haplo-transplant were derived from unmanipulated bone marrow and not involved in the CD34 selection process. All patients in the transplant group received cyclosporin A, mycophenolatemofetil, and short-term MTX as acute graft-versus-host disease (aGVHD) prophylaxis. The details about pre-conditioning regimens and supportive care were described previously [15].

**Definitions and assessments**

CR was defined as bone marrow blasts < 5% with complete hematologic recovery (neutrophil count > 1.0 × 10⁹/L, platelet count > 80 ×10⁹/L, independence of red cell transfusions) and absence of extramedullary disease; Partial remission was defined as bone marrow blasts between 5% and 20% after the first induction cycle; non remission was defined as bone marrow blasts > 20% after the first cycle of induction. Relapse was defined as bone marrow blasts ≥ 5%, the reappearance of blasts in the blood, or development of extramedullary disease. Minimal residual disease (MRD) was assessed by multiparameter flow cytometry (MFC) [16] and PCR-based evaluation of the expression levels of specific fusion genes. MRD-positive was defined as two consecutive positive results by MFC or a specific fusion gene, or both MFC and fusion genes positive in a single sample [15]. Patients with MRD from negative to positive were not classified as having relapsed. Overall survival (OS) was calculated from the date of diagnosis to the date of death from any cause or the date of last contact. EFS was calculated from the
date of diagnosis to the date of first event (relapse, second malignancy, or death due to any cause, whichever occurred first) or the date of last follow-up. When analyzing the OS, EFS, and relapse rate in the haplo-HSCT group, the initial time was calculated from the date of transplantation.

**Statistical analysis**

The last follow-up date was January 01, 2020. Probabilities of OS and EFS were estimated using the Kaplan–Meier method. The cumulative incidence of GVHD, relapse, and non-relapse mortality (NRM) were calculated using competing risk analyses. We used Cox proportional hazards regression to estimate the multivariate hazard ratios (HRs) for OS and EFS and used competing risk regression analyses to estimate the multivariate HRs for relapse. Variables with $P$ values < 0.1 in univariate analysis were included in multivariate analysis. A two-sided $P$ value of 0.05 was considered significant. Data analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL, USA) and SAS (SAS Institute, Cary, NC).

**Results**

**Patient characteristics**

Between January 2013 and December 2017, there were 324 newly diagnosed cases of AML in children. Exclusion criteria eliminated 213 patients with non-FAB-M4/M5 disease, nine patients with FAB-M4/M5 from MDS or CML, 22 patients who failed to complete at least two cycles of chemotherapy, and 10 cases who did not achieve CR1. Based on initial risk stratification criteria, of the remaining 70 evaluable patients, 32 (45.7%) were in the low-risk, 23 (32.9%) were in the intermediate-risk, and 15 (21.4%) were in the high-risk group. Clinical and biological characteristics of patients are summarized in TABLE 1. The 70 patients comprised 21 (30.0%) females and 49 (70.0%) males, with median age at diagnosis of 9 years, of which 33 (47.1%) cases were ≥ 10 years of age. Forty (57.1%) cases were FAB-M4 and 30 were FAB-M5. Eleven (14.3%) patients had central nervous system leukemia at first diagnosis. The median WBC at diagnosis was $47.0 \times 10^9/L$, of which 34 (48.6%) cases had values ≥ $50.0 \times 10^9/L$ and 13 (18.6%) cases had values ≥ $100.0 \times 10^9/L$. The median platelet count at diagnosis was $52 \times 10^9/L$, of which 36 (51.4%) cases had values > $50 \times 10^9/L$. T (16;16), inv 16/CBFB-MYH11 was the most frequent cytogenetic abnormality, which was found in 20 patients (28.6%). Among the detected genetic mutations, $WT1$ had the highest mutation frequency (n = 35, 50.0%), followed by $FLT3-ITD$ (n = 7, 10.0%), $NPM1$ (n = 4, 5.7%), and $CEBPA$ (n = 2, 2.9%). Thirty-three (47.1%) patients received HSCT, of which four were from the low-risk, 18 were from the intermediate-risk group, and 11 were from the high-risk group. The median time from diagnosis to transplantation was 155 days (range, 100–382). Of the 33 patients in the HSCT cohort, three had a 6/6 HLA-matched sibling donor (MSD), and 30 had haplo-HSCT (two with a 5/6 HLA-matched donor, two with a 4/6 HLA-matched donor, and 26 with a 3/6 HLA-matched donor).
Table 1
Clinical and biological characteristics of patients

| Characteristic | Median (range) or N/% |
|----------------|-----------------------|
| Sex           |                       |
| Male          | 49/7                  |
| Female        | 21/3                  |
| Age (years)   |                       |
| ≥ 10          | 33/4                  |
| < 10          | 37/5                  |
| FAB subtypes  |                       |
| M             | 40/5                  |
| M4            | 30/4                  |
| M5            |                       |
| CN SL         | 11/1                  |
| WBC (×10^4)   | 47.0                  |
| (x)           | (1–10)                |
|              | 44                    |
|         |        |        |        |
|---------|--------|--------|--------|
| 9/50    | 3.9    | 34     | 8.6    |
| ≥ 50    |        |        |        |
| < 50    | 36     | 86     |        |
| ≥ 10    | 13     |        | 8.6    |
| < 10    | 57     |        | 1.4    |
| H GB (g/L) |        |        |        |
| ≥ 84    | 35     | 34     | 8.6    |
| < 84    | 0.0    | 0.0    |        |
| PL T (×10^9/L) |        |        |        |
| ≥ 50    | 34     | 8.6    |        |
| ≤ 50    |        |        |        |
| Cy to genetic | 7/10.0 |        |        |
| t (8; 21) |        |        |        |
| RU      | 5.7    |        |        |
| 1/       | 1.4 |
|---|---|
| 5/       | 7.1 |
| 1/       | 1.4 |
| 0/       | 0.0 |
| 4/       | 5.7 |
| 19       |   |
| /2       |   |
| 7.1      |   |
| 9/       |   |
| 12.      |   |
| 9        |   |
| 2/       | 2.9 |
| 2        |   |
| 3        |   |
| Other    |   |
| 1/       |   |
| 2/       |   |
| 3/       |   |
| Other    |   |
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NA
| FL T3 - ITD (+) | 7/10.0 0 |
|-----------------|---------|
| NP M I (+)      | 4/5.7   |
| CE BP A (+)     | 2/2.9   |
| WT1 (+)         | 35/5.0 0 |
| Risk group      | 32/4.0 5.7 |
| Low             | 23/3.0 2.9 |
| Intermediate    | 15/2.0 1.4 |
| High            |         |
| Courses to CR   | 48/6.0 8.6 |
| 1               | 22/3.0 1.4 |
| 2-3             |         |
| HS CT           | 33/4.0 7.1 |
| MS SD           | 3/9.1   |
| HR D            |         |
Clinical outcomes of all patients

Of the 70 patients enrolled, 48 (68.6%) achieved CR after the first cycle of induction therapy, 21 (30.0%) achieved CR after the second cycle of induction therapy, and one (1.4%) achieved CR after three courses of chemotherapy. Relapse occurred in 19 (24.1%) patients, including 18 hematologic relapses and one hematologic combined with extramedullary leukemia relapse. The median time from diagnosis to relapse was 10.3 months (range 3.1–35.2). Ten of the 19 relapsed patients died of disease progression, whereas one is alive with disease and eight are alive without disease in second CR. Up to the last follow-up time, the median follow-up time was 37.1 months (range 4.9–84.0). Twelve patients had died (10 due to relapse and two due to transplant-related mortality). For all 70 patients, the cumulative incidence of relapse (CIR) at 3 years was 27.9 ± 5.2%, the cumulative incidence of NRM at 3 years was 3.2 ± 2.2%, and the probability rates of OS and EFS at 3 years were 85.3 ± 4.3% and 69.0 ± 5.7%, respectively.

Prognostic factor analysis

Relapse
In univariate analysis (Supp. Fig. 1, Supp. TABLE 2), factors affecting relapse included abnormal genetic mutations (with/without *FLT3-ITD*; \( P = 0.002 \)), risk group \( (P = 0.001) \), and courses to CR \( (P = 0.017) \). In multivariate analysis (TABLE 2), the HRs of relapse were \( 0.196 \) (95% CI, 0.065–0.592; \( P = 0.004 \)) for patients with PLT > \( 50 \times 10^9/L \) compared to those with PLT \( \leq 50 \times 10^9/L \) and \( 5.236 \) (95% CI, 1.137–24.109; \( P = 0.034 \)) for patients with *FLT3-ITD* mutation compared to those without *FLT3-ITD* mutation.
### Table 2
Multivariate analysis of relapse, overall survival, and event-free survival (n = 70)

| Variable | OS HR (95% CI) | P | EFS HR (95% CI) | P | CIR HR (95% CI) | P |
|----------|----------------|---|-----------------|---|-----------------|---|
| Male     | 0.4 (0.38–1.38) | 0.15 | 0.1 (0.06–0.49) | 0.04 | 0.0 (0.06–0.493) | 0.01 |
| PLT 50 × 10^9/L | 0.72 (0.050–0.493) | 0.001 | 0.4 (0.016–0.53) | 0.55 | 0.2 (0.062–2.900) | 0.46 |
| With CB | 0.64 (0.056–1.190) | 0.57 | 0.4 (0.245–2.900) | 0.19 | 0.6 (0.219–1.685) | 0.38 |
| FL T3 ITD | 1.1 (0.248–5.301) | 0.34 | 4.9 (1.190–20.568) | 0.02 | 5.2 (1.137–24.109) | 0.03 |
| Low risk | 0.1 (0.02–0.48) | 0.07 | 0.1 (0.035–0.28) | 0.02 | 0.6 (0.058–8.400) | 0.34 |
| One co | 0.5 (0.07–4.8) | 0.48 | 0.5 (0.05–0.74) | 0.1 | 0.5 (0.082–8.190) | 0.19 |
Event-free survival

In univariate analysis (Supp. Fig. 1, Supp. TABLE 2), factors influencing EFS included cytogenetic abnormality (with/without \( CBF; P = 0.023 \)), abnormal genetic mutation (with/without \( FLT3-ITD; P < 0.001 \)), risk group \( (P < 0.001) \), and courses to CR \( (P = 0.007) \). In multivariate analysis (TABLE 2), the HRs of EFS were 0.172 (95% CI, 0.060–0.493; \( P = 0.001 \)) for patients with PLT > 50 × 10^9/L compared to those with PLT ≤ 50 × 10^9/L, 4.947 (95% CI, 1.190–20.568; \( P = 0.028 \)) for patients with \( FLT3-ITD \) mutation compared to those without \( FLT3-ITD \) mutation, and 0.135 (95% CI, 0.036–0.505; \( P = 0.002 \)) for patients in the low-risk group compared to those in intermediate- and high-risk groups.

Overall survival

In univariate analysis (Supp. Fig. 1, Supp. TABLE 2), factors influencing OS included abnormal genetic mutations (with/without \( FLT3-ITD; P = 0.018 \)), risk group \( (P = 0.001) \), and courses to CR \( (P = 0.029) \). In multivariate analysis (TABLE 2), the HR of OS was 0.102 (95% CI, 0.021–0.496; \( P = 0.007 \)) for patients in the low-risk group compared to those in intermediate- and high-risk groups.

Effect of transplant on intermediate- and high-risk group

To explore the effect of transplant in intermediate- and high-risk groups, we further performed univariate and multivariate analyses of these patients. In intermediate- and high-risk groups, compared to that in the chemotherapy group, HSCT resulted in significantly higher EFS (3 years; 66.9% ± 9.2% vs. 11.1% ± 10.5%; \( P < 0.001 \)) and lower CIR (3 years; 24.7% ± 7.9% vs. 92.1% ± 7.5%; \( P < 0.001 \); Fig. 2, TABLE 3). In multivariable analysis, HSCT was also independently associated with superior EFS (HR: 0.058, 95% CI: 0.016–0.212, \( P < 0.001 \)) and lower CIR (HR: 0.040, 95% CI: 0.013–0.129, \( P < 0.001 \); TABLE 3). Moreover, univariate analysis showed that risk group (intermediate- or high-risk) was a factor influencing OS \( (P = 0.026) \), EFS \( (P = 0.013) \), and CIR \( (P = 0.026) \), whereas PLT \( (\geq 70 \times 10^9/L \text{ or not}) \) and abnormal genetic mutations (with/without \( FLT3-ITD \)) were factors influencing EFS \( (P = 0.016, P = 0.025, \text{respectively}) \) and CIR \( (P = 0.023, P = 0.019, \text{respectively}; \text{TABLE 3}) \). FAB subtype (AML-M4 or M5) was a factor influencing CIR \( (P = 0.041; \text{TABLE 3}) \). Multivariate analysis showed that PLT \( (\geq 70 \times 10^9/L \text{ or not}) \) was a prognostic factor of EFS \( (HR: 0.274, 95\% \text{ CI: } 0.085–0.882, P = 0.030) \) and CIR \( (HR: 0.267, 95\% \text{ CI: } 0.073–0.969, P = \)
0.044), whereas risk group (intermediate- or high-risk) was a prognostic factor of OS (HR: 4.127, 95% CI: 1.065–16.003, \( P = 0.040 \)) and EFS (HR: 4.115, 95% CI: 1.013–16.706, \( P = 0.048 \); TABLE 3).
Table 3
Univariate analysis and multivariate analysis of long-term survival in intermediate- and high-risk group (n = 38)

| Factors | 3-y OS | 3-y EFS | 3-y CIR |
|---------|--------|---------|--------|
|         | Univariate (P) | Multivariate (P) | Univariate (P) | Multivariate (P) |
|         | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) |
| Sex     | 0.4    | 0.5     | 0.7    | 0.7     |
| Age     | 0.2    | 0.6     | 0.7    | 0.4     |
|         | 17     | 11      | 43     | 80      |
| FAB Subtype | 0.4   | 0.2     | 0.0    | 0.0     |
|         | 31     | 98      | 30     | 41      |
|         | 0.041  | 0.471   | 0.030  | 0.211   |
|         | 0.156  | 1.467   | 0.0267 | 0.0411  |
| WBC     | 0.5    | 0.3     | 0.6    | 0.6     |
|         | 0.90   | 0.21    | 0.74   | 0.75    |
|         | 0.085  | 0.082   | 0.023  | 0.044   |
|         | 0.073  | 0.969   | 0.310  | 0.310   |
| HGB     | 0.1    | 0.0     | 0.0    | 0.0     |
|         | 0.19   | 0.016   | 0.026  | 0.030   |
|         | 0.005  | 0.008   | 0.002  | 0.002   |
| PLT     | 0.1    | 0.4     | 0.0    | 0.0     |
|         | 0.75   | 0.25    | 0.5    | 0.5     |
|         | 0.41   | 0.25    | 0.74   | 0.74    |
|         | 0.27   | 0.27    | 0.067  | 0.067   |
|         | 0.005  | 0.008   | 0.004  | 0.004   |
| FLOT3 - ITD | 0.4  | 0.2     | 0.4    | 0.4     |
|         | 0.08   | 0.2     | 0.0    | 0.0     |
|         | 0.04   | 0.0     | 0.026  | 0.026   |
|         | 0.005  | 0.008   | 0.002  | 0.002   |
| Risk gr | 0.4    | 0.8     | 0.0    | 0.0     |
|         | 0.80   | 0.93    | 0.48   | 0.48    |
|         | 0.04   | 0.026   | 0.016  | 0.016   |
Clinical outcomes of haplo-HSCT cohort

Of the 30 patients in the haplo-HSCT cohort, all survived beyond day 28, except one recipient who died of intracranial infection 8 days post-transplantation. The median post-transplant observation time was 30.0 months (range, 0.3–77.4). Neutrophil engraftment (> 0.5 × 10^9/L for 3 consecutive days) occurred in 29 patients at a median of 13 days (range, 10–20). Platelet engraftment (> 20 × 10^9/L without transfusion for 7 consecutive days) occurred in 28 patients at a median of 14 days (range, 7–35). A total of 21 (70.0%) patients developed aGVHD with a median time from HSCT to aGVHD of 15 days (range, 10–48). The cumulative incidences of grade II-IV and III-IV aGVHD at day 100 were 35.8 ± 8.0% and 9.8 ± 5.7%, respectively. Sixteen (53.3%) patients developed chronic GVHD (cGVHD) with a median time from HSCT to cGVHD of 195 days (range, 102–647). The cumulative incidences of cGVHD and extensive cGVHD at 3 years were 56.8 ± 7.6% and 16.4 ± 3.2%, respectively. Nineteen (63.3%) patients developed cytomegaloviremia, and the cumulative incidences of cytomegaloviremia at day 100 after HSCT was 64.9 ± 5.7%. Seven (23.3%) patients experienced hemorrhage cystitis, and the cumulative incidences of total hemorrhage cystitis and grade III-IV hemorrhage cystitis at day 100 after HSCT were 28.7 ± 6.2% and 6.7 ± 1.4%, respectively. During the follow-up period after HSCT, seven patients experienced relapse with a median time from HSCT to relapse of 243 days (range, 80–824). The CIR at 3 years was 24.6 ± 7.6% (95% CI: 13.4–45.0%). Two patients died due to non-relapse factors after transplantation, one died of multiple organ failure 235 days post-transplantation, and one died of intracranial infection 8 days post-
transplantation. The NRM at 3 years was 6.6 ± 4.1% (95% CI: 1.9–22.6%). At the time of the last follow-up, seven patients (five due to relapse and two due to transplant-related mortality) had died with a median time to death of 235 days (range, 8–885). The probability rates of OS and EFS at 3 years after HSCT for all 30 children were 74.2 ± 8.6% and 68.3 ± 8.9%, respectively (Supp. Fig. 2).

Discussion

Whether it was research on clinical efficacy or molecular biology, previous studies have mainly focused on overall pediatric AML. However, research on pediatric FAB-M4/M5, a clinically and biologically heterogeneous disease, is hardly reported. Here, we provide the clinical characteristics and molecular genetics of pediatric FAB-M4/M5 and explored the efficacy of HSCT, especially haplo-HSCT, for these patients. FAB-M4/M5 comprises 31.5% of total childhood AML in our center. Nearly half of the patients were older than 10 years and had a WBC count more than 50 × 10^9/L at diagnosis. Further, 14.3% of patients had CNSL. These clinical features are consistent with previous reports [3, 8, 17]. In this retrospective study, the 3-year OS and EFS of children with FAB-M4/M5 were 85.3% and 69.0%, respectively, which were similar to results reported by Imamura et al [18] and also similar to the results of overall pediatric AML reported in other centers [4, 18–20].

The significant improvements in the prognosis of pediatric AML are mainly attributed to refinements in cytogenetics-based risk stratification and subsequent risk-directed therapy. Therefore, comprehensive molecular profiling at diagnosis is essential. Existing evidence has shown the precise prognostic significance of some certain gene fusions and mutations [10, 20–23]. Including CBF, NPM1, and CEBPA mutations, the consensus low-risk group currently accounts for approximately 30% to 40% of overall pediatric AML and is associated with a favorable prognosis [9, 10, 14, 24]. Due to the more frequent occurrence of t(16;16), inv 16/CBFB-MYH11 in pediatric FAB-M4/M5 [25], the proportion of FAB-M4/M5 patients in the low-risk group might be much higher than that of overall childhood AML. The low-risk group comprised 47.2% of all children with FAB-M4/M5 in this study and had an excellent prognosis with 3-year OS and EFS probabilities of 96.9% and 87.3%, respectively. As a common marker of the high-risk group, FLT3/ITD mutations occur in 10% to 20% of children with AML [14] and often confer poor survival [12, 26]. We found a similar result in our study cohort. Patients with FLT3/ITD mutations accounted for 10.0% of overall FAB-M4/M5 cases and these were identified as an independent risk factor for relapse and survival.

We found that in intermediate- and high-risk groups, HSCT showed demonstrable superiority in terms of EFS and CIR over chemotherapy. According to the combined data from four cooperative group clinical trials [27], compared to those with chemotherapy alone, allogeneic SCT from a matched related donor conferred survival advantages to pediatric patients with intermediate-risk AML (8-year DFS, 39 ± 5% vs. 58 ± 7%; 8-year OS, 51 ± 5% vs. 62 ± 7%; P < 0.01). Accordingly, another clinical trial by the Japanese Childhood AML Cooperative Study Group also observed a significant difference in 5-year DFS for intermediate-risk patients in the matched related HSCT group and the chemotherapy group (5-year DFS, 81.8% vs. 52.9%; P = 0.029), but there was no significant difference in OS [20]. Burke et al [28] and
Hyakuna et al [29] reported the excellent effect of allogeneic HSCT for children with high-risk AML, and their results showed that the high-risk group could benefit from HSCT. In addition, haplo-HSCT was also found to have great promise for the treatment of children with FAB-M4/M5 in the current study. The long-term survival and transplantation-related mortality achieved in the haplo-HSCT group were comparable to those reported in patients who underwent MSD HSCT or matched unrelated donor HSCT reported in other studies [4, 28]. Thus, we recommend that haplo-HSCT can be a viable alternative treatment for children with intermediate- and high-risk FAB-M4/M5 who do not have a matched donor available.

We also provide several possible causes of deviations in the results and limitations. First, the frequency of HSCT during CR1 in our study was similar to that in the study of Horan et al [27] but was much higher than that at other centers, which might lead to bias [18, 30]. Second, although HSCT showed obvious advantages for the high-risk group, the survival rate of these patients was not good. We consider that the small number of cases in the high-risk group available for this analysis limited the ability to draw any definitive conclusions, but our results do suggest that even with HSCT, these patients have poor outcomes. Finally, due to the high salvage rate of relapsed patients in the chemotherapy cohort, there was no statistical significance in the OS rate between the HSCT and chemotherapy cohorts in the intermediate- and high-risk groups. Therefore, we wonder if it might be advantageous to reserve HSCT for the treatment of relapsed patients, which could avoid the risk of unnecessary HSCT and maximize the efficacy of HSCT. Of course, the results in this study need to be validated by large-scale multicenter prospective studies.

**Abbreviations**

*aGVHD*

*acute graft-versus-host disease*

*AML*

acute myeloid leukemia

*ATG*

anti-human thymocyte immunoglobulin

*BU/CY*

busulfan-cyclophosphamide

*CBF*

core-binding factor

*cGVHD*
chronic graft-versus-host disease

CIR

cumulative incidence of relapse

CML

chronic myelogenous leukemia

CNSL

central nervous system leukemia

CR1

first complete remission

EFS

event-free survival

FAB

French-American-British

FLT3

FMS-like tyrosine kinase 3

haplo-HSCT

haploidentical HSCT

HRs

hazard ratios

HSCT

hematopoietic stem cell transplantation

MDS

myelodysplastic syndrome

Me-CCNU
methyl-N-(2-chloroethyl)-N-cyclohexyl-N-nitrosourea

*MFC*

multiparameter flow cytometry

*MRD*

minimal residual disease

*MSD*

matched sibling donor

*MTX*

methotrexate

*NRM*

non-relapse mortality

*OS*

overall survival

*WBC*

white blood cell count

**Declarations**

**Ethics approval and consent to participate:**

Written informed consent for publication of their clinical details was obtained from the patients’ guardians. A copy of the consent form is available for review by the Editor of this journal. The research protocol was approved by the ethical committee of the Peking University People’s Hospital.

**Conflict of Interest Statement:**

The authors declare that they have no conflict of interest.

**Data available statement:**
All data generated or analysed during this study are included in this published article and its supplementary information files.

Authors’ contributions:

YJX and PS analyzed the data, wrote the manuscript, and contributed equally to this work; YFC, ADL and YW were responsible for editing and reviewing this manuscript. YXZ and JW organized and summarized the clinical data. LPZ and XJH designed the research, and were the chief persons in charge of the manuscript. All authors provided the approval of the final manuscript for submission.

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Figures
FIGURE 1. Overall profile of the enrolled patients including risk classification and donor availability.

Abbreviations: chemo, chemotherapy; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; chemo, chemotherapy; CR, complete remission; LR, low-risk; IR, intermediate-risk; HR, high-risk; HSCT, hematopoietic stem-cell transplantation; HRD, haploidentical related donor; MSD, matched sibling donor.

Figure 1

Overall profile of the enrolled patients including risk classification and donor availability
**FIGURE 2.** The EFS and CIR of the HSCT group and chemotherapy group in patients with intermediate- and high-risk FAB-M4/M5 disease. A: EFS. B: CIR.

**Abbreviations:** EFS, event-free survival; CIR, cumulative incidence of relapse; HSCT, hematopoietic stem-cell transplantation.

Figure 2
The EFS and CIR of the HSCT group and chemotherapy group in patients with intermediate- and high-risk FAB-M4/M5 disease. A: EFS. B: CIR

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