Impact of pre-transplantation minimal residual disease (MRD) on the outcome of Allogeneic hematopoietic stem cell transplantation for acute leukemia

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\textbf{ABSTRACT}

\textbf{Objective:} To investigate the impact of minimal residual disease (MRD) before allogeneic hematopoietic stem cell transplantation (allo-HSCT) on the outcome of acute leukemia.

\textbf{Methods:} Data from 114 patients who were diagnosed with acute leukemia (AL) and underwent allo-HSCT between Jan 2013 and Dec 2019 were collected and analyzed. The patients were attributed into MRD positive (MRD+) group and MRD negative (MRD−) group.

\textbf{Results:} Among the 114 acute leukemia patients, there were 32 MRD+ patients before transplantation, and 82 MRD− patients. No significant difference was found between the MRD+ group and the MRD− group in the incidence of acute graft-versus-host disease (aGvHD) ($p = 0.09$). Compared with the MRD+ group, the MRD− group had a higher incidence of chronic graft-versus-host disease (cGvHD) ($p = 0.008$). There is no significance in relapse between the two groups ($p = 0.084$), while the incidence of relapse was seemingly higher in the MRD+ group: 36.9% Vs 19.7%. We attributed to the lack of sample size and NRM in MRD+ group was remarkably higher. The MRD+ group had significantly worse one-year overall survival (OS) ($p = 0.003$) and one-year progression-free survival (PFS) ($p = 0.009$). In the multivariate analysis, MRD+ was an independent prognostic factor for OS (HR = 1.898; 95%CI 1.042–3.457; $p = 0.036$).

\textbf{Conclusion:} Pre-transplantation MRD positive status is a risk factor for survival and prognosis after HSCT. Upon this, emphasis should be put on (1) screening more efficient chemo regimen with targeted agents, to help patients reach and keep MRD− status before transplantation; (2) designing better management with different GvHD prophylaxis treatment, timely disease monitoring and preemptive intervention on relapse.

\textbf{Introduction}

Acute leukemia (AL) represents a heterogeneous group of hematological malignancies with varying clinical, morphological, immunological and molecular characteristics [1]. At present, with the widespread use of traditional chemotherapy regimens combined with targeted agents, the prognosis of patients with AL has been improved significantly. But patients with refractory or relapsed AL still have an extremely poor prognosis [2,3]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a crucial treatment option for patients with AL, especially for that primary chemo-refractory disease and early relapse after standard chemotherapy [4,5]. However, even after HSCT relapse is still the major hurdle and leads to poor prognosis.

Minimal residual disease (MRD) refers a state in which traces of leukemia cells could not be detected using morphologic methods when patients in complete remission (CR), while using PCR or flow cytometry leukemia tumor load could still be detected. In recent years, MRD has been widely used in clinical monitoring and an indication of relapse of all kinds of malignant hematological diseases, which has a certain guiding significance for disease risk stratification and chemotherapy scheme selection [6,7]. We retrospectively analyzed the basic clinical characteristics, post-transplantation complications, relapse and survival for 114 patients with AL who underwent allo-HSCT in the department of hematology in Jiangsu Province Hospital, Jiangsu Province P.R. China. The impact of pre-transplantation MRD status on the outcome of allo-HSCT was also addressed.

\textbf{Materials and methods}

\textbf{Patients}

A total of 114 patients with AL who underwent allo-HSCT in the department of hematology in Jiangsu...
Province Hospital from Jan 2013 to Dec 2019 were enrolled in this study. All patients achieved morphological CR status according to the criteria of NCCN (National comprehensive cancer network) [2] and were arranged to detect MRD status with bone marrow routinely before transplantation. Patients were divided into two groups according to the results of the MRD test before transplantation, including the MRD positive group (MRD+) and MRD the negative group (MRD−). This study was approved by the Medical Ethical Committee of the First Affiliated Hospital of Nanjing Medical University.

Detection of MRD

At diagnosis of AL, leukemia-associated aberrant immunophenotypes (LAIP) were identified by multiparametric flow cytometry (MFC) and all patients had at least one useful phenotype for follow-up throughout leukemia treatment. All patients underwent bone marrow aspiration within one month before transplantation and 2 mL Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant bone marrow samples were collected for MRD detection by flow cytometry. The following monoclonal antibodies were used to track LAIP by eight-color MFC: CD2, CD3, CD7, CD5, CD13, CD14, CD15, CD19, CD33, CD34, CD10, CD20, CD45, CD61, CD22, CD117 and HLA-DR. Flow cytometry and fluorescent labeled antibodies were produced by BD Biosciences company (Becton Dickinson, Franklin Lakes, NJ, USA). Hemolysin and cleaning solution were purchased from Beckman Company in Germany. CellQuest software (BD Biosciences) was used for data analysis. At least 5 × 10⁶ events were measured with each sample. MRD ≥ 0.01% was defined as positive (MRD+) and MR < 0.01% was considered as MRD negative (MRD−).

Conditioning regimens

All of the patients enrolled were fit for the myeloablative conditioning regimen and the standard Bu/Cy regimen was chosen as busulfan 3.2 mg kg⁻¹ day⁻¹ (from day -7 to -4) combined with cyclophosphamide 60 mg kg⁻¹ day⁻¹ (day -3 to -2). The patient will not receive any chemical toxic therapy and have a rest on day 1. The stem cells were infused intravenously on day 0. The minimum cell counts are defined as 5 × 10⁹/Kg for total nucleated cell (TNC) and 2 × 10⁶/Kg for CD34⁺ cell.

Evaluation of the engraftment and chimerism

The definition for neutrophil engraftment is neutrophil counts more than 0.5 × 10⁹/L in consecutive 3 days, and the platelet engraftment is considered as platelet counts exceed 20 × 10⁹/L without platelet transfusion in consecutive 7 days. Bone marrow aspiration was routinely performed on day 30, 60, 90, 180, 270 and 360 after the HSCT. Hematopoietic chimerism was identified by sex chromosome determination using the FISH (fluorescence in situ hybridization) method if the donor and recipient were sex-mismatched or short tandem repeats (STR) by PCR (polymerase chain reaction) technique if they were in the same gender group.

Immunosuppression and management

The prophylaxis treatment of graft-versus-host disease (GvHD) for matched sibling donors (MSD) is mainly based on cyclosporine A (minimum concentration ranging from 200–400 ng/mL) combined with short course methotrexate (methotrexate 15 mg/m² on day 1 and 10 mg/m² for day 3, 6 and 11). The strategies for haploidentical stem cell transplantation include cyclosporine A, short-term methotrexate, mycophenolate mofetil, basiliximab and anti-thymocyte globulin (ATG, Rabbit Anti-human Thymocyte Globuli, Genzyme Polyclonals S.A.S), in which the doses of cyclosporine and methotrexate are as same as MSD transplantation, and ATG was administrated at 2.5 mg/kg on day -5 to -2, basiliximab 20 mg on day 0, and the oral mycophenolate mofetil (MMF) dose was 500 mg twice a day on day -1 and was tapered off after 28 days if no acute graft-versus-host disease (aGvHD) was observed. We graded each aGvHD patient using the MAGIC consortium [8].

Supportive care

All patients received central venous catheters and were isolated in a laminar airflow room. Empirical antibiotics and anti-fungal agents were administered to the patients when they experienced fever. The cytomegalovirus (CMV) and Epstein–Barr virus (EBV) were measured weekly during the immunosuppressive period and the patients were administered with gancyclovir when CMV was positive. Trimethoprim/sulfamethoxazole was given for the prevention of pneumocystis carinii infection. Irradiated blood components were infused while needed to maintain the hemoglobin above 70 g/L and platelet counts over 20 × 10⁹/L. The subcutaneous G-CSF (5 μg kg⁻¹ day⁻¹) was administered to all recipients from the day 5 after transplantation until neutrophil recovery. All patients were transfused with 10 g of gamma-immunoglobulin each week from transplantation day to 3 months after transplantation.

Statistical analysis

The research data were processed by Statistical Product and Service Solutions (SPSS) (Version 26.0)
software package. The measurement data were tested by an independent sample t-test, and the rates were compared by the chi-square test. Kaplan–Meier method was used to draw the survival curve, and Log-Rank was used to test whether there was a statistical difference between the curves. The difference was statistically significant (p < 0.05). Overall survival (OS) is defined as the time from post-transplant to death and progression-free survival (PFS) is considered as the time when the disease progressed for the first time or died of any cause after transplantation.

Results

Patient characteristics

Among the 114 patients with AL, the median age was 39 years old (range from 11y to 60y). The number of ALL and AML patients was 43 (37.7%) and 71 (62.3%) separately. Ninety-seven (85.1%) cases were in the first complete remission (CR1) status and the other 17 (14.9%) cases were in the second complete remission or the third remission (CR2≥2) status. A total of 64 patients underwent haploidentical transplantation, and 50 patients experienced MSD transplantation. The comparative results for patient, disease and transplant-related characteristics between the two groups were summarized in Table 1.

Stem cell engraftment

In the MRD+ group, neutrophil engraftment was successful in all patients, while two patients failed to get platelet engraftment. The median engraft time of neutrophils and platelets were 12 days (9–22 days) and 13 days (10–32 days), respectively. In the MRD− group, there were one patient and 6 patients failed in neutrophil and platelet engraftment, respectively. The median engraftment time were 14 days (8–25 days) for neutrophils, and 15 days (5–35 days) for platelets. No significant difference was found in neutrophil engraftment time and platelet engraftment time between the MRD+ group and MRD− group (p > 0.05) (Table 2).

Evaluation of early complications after transplantation

A total of 64 (56.1%) patients developed a GvHD, and 35 (30.7%) patients suffered from chronic graft-versus-host disease (cGvHD). In the MRD+ group, there were 22 (68.8%) cases of aGvHD, including 4 cases of grade III-IV aGvHD. In the MRD− group, there were 42 (51.2%) cases of aGvHD, including 12 cases of grade III-IV aGvHD. The cumulative incidence of grade III-IV acute GvHD was 14.6% in the MRD− group, 12.5% in the MRD+ group (p = 0.78). However, there was a significant difference in the incidence of cGvHD between the two groups (12.5% in MRD+ group Vs 37.8% in MRD− group, p = 0.008) (Table 2).

Prognostic impact of MRD

Of the 32 patients in the MRD+ group, 21 patients died and 11 patients survived during the follow-up period. Among all the 21 mortality, 9 patients died of relapse and 12 patients died of non-relapse causes. 27 out of 82 cases in the MRD− group were dead, including 9 patients died of relapse. The one-year OS of the MRD − group and MRD+ group were 70.2% and 46.9%, respectively; and the one-year PFS was 64.3% and 43.8%, respectively. There was a significant difference in OS between the two groups (p = 0.003, Figure 1 (A)). And the same result was found in PFS. (p = 0.009, Figure 1(B).

In the MRD+ group, 10 patients relapsed after transplantation, of which 9 patients died after relapse, and one patient survived during the follow-up period. The one-year cumulative relapse rate was 25.2% and the 3-year cumulative relapse rate was 36.9%. In the MRD− group, 14 patients relapsed after transplantation, of which 9 patients died after relapse, and 5 patients survived during the follow-up period. The 1-year cumulative relapse rate was 17.9% and the 3-

Table 1. Clinical characteristics of 114 patients before transplantation.

| Clinical features                  | MRD+ (N = 32) | MRD− (N = 82) | p-value |
|-----------------------------------|---------------|---------------|---------|
| Gender                            |               |               | 0.105   |
| Male                              | 11 (34.4%)    | 42 (51.2%)    |         |
| Female                            | 21 (65.6%)    | 40 (48.8%)    |         |
| Age                               | 40 (13–64)    | 38 (11–65)    | 0.365   |
| Disease                           | 8 (25%)       | 35 (42.7%)    | 0.08    |
| ALL                               | 24 (75%)      | 47 (57.3%)    |         |
| Remission state                   | 25 (78.1%)    | 72 (87.8%)    | 0.192   |
| CR1                               | 7 (21.9%)     | 10 (12.2%)    |         |
| CR2                              |               |               |         |
| CR2≥2                             |               |               |         |
| Transplant type                   | 19 (59.4%)    | 45 (54.9%)    | 0.664   |
| Haplo-HSCT                        | 13 (40.6%)    | 37 (45.1%)    |         |
| Number of infusied cells          |               |               |         |
| CD34 (10^6/kg)                    | 5.74 (2.4–14.1) | 5.03 (1.0–44.5) | 0.082   |
| MNC (10^6/kg)                     | 10.2 (3.7–20.0) | 8.2 (3.4–23.25) | 0.072   |

Note: CR, complete remission; Haplo-HSCT, haploidentical hematopoietic stem cell transplantation; MSD-HSCT, matched sibling donor hematopoietic stem cell transplantation; MNC, mononuclear cell.

Table 2. Comparison of observation indexes after transplantation.

| Cell engraftment time | MRD+ (N = 32) | MRD− (N = 82) | p-value |
|-----------------------|---------------|---------------|---------|
| Neutrophils (day)     | 12 (9–22)     | 14.5 (8–25)   | 0.672   |
| Platelets (day)       | 13 (10–32)    | 15 (5–35)     | 0.708   |
| cGVHD                 | 26            | 73            | 0.270   |
| aGVHD                 | 22 (68.8%)    | 42 (51.2%)    | 0.090   |
| IIIaGVHD              | 18(56.3%)     | 30(36.6%)     |         |
| IIIaGVHD              | 4(12.5%)      | 12(14.6%)     |         |
| cGVHD                 | 4 (12.5%)     | 31 (37.8%)    | 0.008   |

Note: GVHD, graft-versus-host disease; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.
year cumulative relapse rate was 19.7%. No significant difference was found in the cumulative relapse rate between the two groups (p = 0.084, Figure 1(C). The cumulative incidence of NRM in the MRD− group did differ from that in the MRD+ group (20.5% in MRD− group Vs 39.7% in MRD+ group, p = 0.046, Figure 2(A).

Graft-versus-host disease-free, relapse-free survival (GRFS) in the MRD+ group and MRD− group did differ significantly (46.4% in MRD− group Vs 26.5% in MRD+ group, p = 0.005, Figure 2(B).

Multivariate analysis of the prognostic impact of MRD

The COX regression model was used to analyze the related factors of OS. Univariate Cox regression analysis revealed that age >50y, MRD+, relapse and Haplo-HSCT were associated with higher mortality. Upon multivariable adjustment, age>50y (HR = 2.254; 95%CI 1.203–4.222; p = 0.011), MRD+ (HR=1.898; 95%CI 1.042–3.457; p = 0.036), relapse(HR = 2.447; 95%CI 1.320–4.535; p = 0.004) and Haplo-HSCT(HR = 2.358; 95%CI 1.270–4.376; p = 0.007) were significantly associated with poor survival (Table 3).

Discussion

Acute leukemia is a common kind of hematological malignant tumor. Although low-risk patients can obtain a longer survival time and good prognosis from intensive chemotherapy, the only choice for high-risk patients to prolong survival time is HSCT. Relapse is the major reason that limits the efficacy of HSCT and leads to the failure of transplantation. Residual leukemia cells in patients are the main cause of disease relapse. The OS and PFS for no remission (NR) patients are significantly lower than those for CR patients, and the cumulative relapse rate is also significantly higher than that of remission patients [9,10]. However, even if the patient is in CR status, the MRD may be detected in some patients. MRD can be detected by flow cytometry (FCM), polymerase chain reaction (PCR), real-time quantitative polymerase chain reaction (RQ-PCR), reverse transcriptase polymerase chain reaction (RT-PCR) or next generation sequencing (NGS) [11,12]. The detection of MRD is critical for predicting the outcome of transplantation and for selecting the intensity of further treatment strategy [13–15].

In our study, no significance in the incidence of aGvHD (p = 0.090) between MRD + and MRD− group. But there was a significant difference in the incidence of cGvHD between the two groups (p = 0.008). It might be due to the longer OS in the MRD− group compared with the MRD+ group. The median OS of the MRD+ group was significantly shorter than that of the MRD− group (p = 0.003). The results of the COX multivariate survival analysis showed that the survival risk of MRD− was lower than that of MRD+ (p = 0.032). This is similar to the

![Figure 1](image1.png)

**Figure 1.** Kaplan–Meier curves of (A) overall survival (OS), (B) progression-free survival (PFS), (C) cumulative incidence of relapse between the MRD+ group and MRD− group.

![Figure 2](image2.png)

**Figure 2.** Kaplan–Meier curves of (A) non-relapse mortality (NRM), (B) GVHD/Relapse-Free survival (GRFS) between the MRD+ group and MRD− group.
Tables 3. Analysis of risk factors for OS.

| Risk factors | Univariate analysis | Multivariate analysis |
|--------------|---------------------|----------------------|
|              | p-value HR | HR | p-value HR | HR |
| Gender       | 0.322 | 0.751 | (0.426–1.325) | 0.332 | 0.748 | (0.425–1.331) |
| Age          | 0.144 | 1.017 | (0.994–1.041) | 0.144 | 1.017 | (0.994–1.041) |
| Pre-MRD      | 0.004 | 2.335 | (1.312–4.156) | 0.032 | 1.921 | (1.056–3.495) |
| Remission rate | 0.44 | 1.332 | (0.643–2.760) | 0.44 | 1.332 | (0.643–2.760) |
| Disease (AML or ALL) | 0.164 | 0.642 | (0.344–1.198) | 0.164 | 0.642 | (0.344–1.198) |
| Donor        | 0.015 | 2.138 | (1.157–3.951) | 0.015 | 2.138 | (1.157–3.951) |
| The number of CD34+ | 0.298 | 0.960 | (0.888–1.037) | 0.298 | 0.960 | (0.888–1.037) |
| The number of TNC | 0.502 | 0.977 | (0.914–1.045) | 0.502 | 0.977 | (0.914–1.045) |
| Relapse      | 0.001 | 2.643 | (1.465–4.771) | 0.004 | 2.491 | (1.344–4.617) |

Note: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MNC, mononuclear cell.

All of these studies suggested that MRD is indeed an important factor in relapse and survival, the higher level of MRD, might be a sign of worse prognosis. It is necessary to continue chemotherapy for MRD high-level positive patients to make them negative before transplantation, or could we ignore the low level of MRD since it does not affect OS and PFS? For some refractory cases, MRD+ might be the best statue before treatment, they may have no time for further chemotherapy before transplantation. Thus for these high-risk MRD+ patients, we could also design better management with different GvHD prophylaxis treatment, timely disease monitoring and preemptive intervention on relapse. This is still a controversial issue and more objectives are needed to expand this study.

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