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Prognostic implication of gene mutations on overall survival in the adult acute myeloid leukemia patients receiving or not receiving allogeneic hematopoietic stem cell transplantations

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ABSTRACT

Several gene mutations have been shown to provide clinical implications in patients with acute myeloid leukemia (AML). However, the prognostic impact of gene mutations in the context of allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains unclear. We retrospectively evaluated the clinical implications of 8 gene mutations in 325 adult AML patients; 100 of them received allo-HSCT and 225 did not. The genetic alterations analyzed included *NPM1*, *FLT3*-ITD, *FLT3*-TKD, *CEBPA*, *RUNX1*, *RAS*, *MLL*-PTD, and *WT1*. In patients who did not receive allo-HSCT, older age, higher WBC count, higher lactate dehydrogenase level, unfavorable karyotype, and *RUNX1* mutation were significantly associated with poor overall survival (OS), while *CEBPA* double mutation (*CEBPA*$^{\text{double-mut}}$) and *NPM1*$^{\text{mut}}$/*FLT3*-ITD$^{\text{neg}}$ were associated with good outcome. However, in patients who received allo-HSCT, only refractory disease status at the time of HSCT and unfavorable karyotype were independent poor prognostic factors. Surprisingly, *RUNX1* mutation was an independent good prognostic factor for OS in multivariate analysis. The prognostic impact of *FLT3*-ITD or *NPM1*$^{\text{mut}}$/*FLT3*-ITD$^{\text{neg}}$ was lost in this group of patients receiving allo-HSCT, while *CEBPA*$^{\text{double-mut}}$ showed a trend to be a good prognostic factor. In conclusion, allo-HSCT can ameliorate the unfavorable influence of some poor-risk gene mutations in AML patients. Unexpectedly, the *RUNX1* mutation showed a favorable prognostic impact in the context of allo-HSCT. These results need to be confirmed by further studies with more AML patients.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous neoplasm with great variability in the pathogenesis, response to therapy, and clinical outcome [1]. Thus, individualized treatment strategy according to patients' risk and prognostic factors is the key to achieve better survival. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most powerful treatment modalities, but it also brings considerable treatment related risks to AML patients. Hence, careful selection of patients who may benefit from allo-HSCT is essential to get the best survival results [2].

Several gene mutations were shown to have clinical implications in AML patients [3–5], especially in those with normal karyotype [6–8]. Yet, the prognostic relevance of these gene mutations was derived without taking the effect of HSCT into account. Cytogenetic risk factors have been routinely used as guides to choose proper AML patients for allo-HSCT [9,10]. Recent reports suggested that gene mutations might also have similar prognostic implications in this aspect. Cytogenetically normal AML patients with mutant *CEBPA* or mutant *NPM1* but without *FLT3*-ITD (*NPM1*$^{\text{mut}}$/*FLT3*-ITD$^{\text{neg}}$) have more favorable prognosis [11], but these patients do not benefit from allo-HSCT in the first complete remission (CR) when compared with traditional consolidation chemotherapy alone [11]. In contrast, allo-HSCT was shown to have a beneficial effect in AML patients with other genotypes including *FLT3*-ITD or *NPM1*$^{\text{wild}}$/*FLT3*-ITD$^{\text{neg}}$. However, different result was reported in patients with *FLT3*-ITD. [12] Interestingly,

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a report showed that *RUNX1* mutation, a poor-risk genotype [13], was associated with better relapse-free survival in patients receiving allo-HSCT [14]. Nevertheless, the available literature concerning clinical implications of gene mutations in AML patients receiving all-HSCT is still very limited. Therefore, we investigated the prognostic implication of gene mutations on clinical outcomes in this clinical epidemiologic study by comparing survival between patients with and without gene mutations among 100 AML patients who received allo-HSCT and 225 others who did not.

2. Subjects and methods

2.1. Patients

This study was approved by the Institutional Review Board of the National Taiwan University Hospital (NTUH) in advance. The written informed consents were obtained from all participants. A total of 542 patients with newly diagnosed *de novo* AML at the NTUH from 1995 to 2007 were screened for gene mutations. Pediatric patients less than 16 years of age and patients with acute promyelocytic leukemia were excluded from this study. And, 138 AML patients who received only palliative or supportive cares due to comorbidity or based on patient's own decision were not included in analysis. Finally, 325 AML patients who received conventional induction chemotherapy (idarubicin 12 mg/m^2 per day on days 1–3 and cytarabine 100 mg/m^2 per day on days 1–7) were recruited into this study. Treatment response was defined according to the criteria of Cheson et al. [15]. Among the 325 recruited AML patients, 100 received allo-HSCT (the allo-HSCT group) and 225 did not (the non allo-HSCT group). For patients who did not received allo-HSCT in first complete remission, 3 to 4 courses of consolidation chemotherapy with high dose cytarabine (2 g/m^2 q12 h for 4 days) with or without an anthracycline (idarubicin or mitoxatrone) were given after achievement of complete remission [16,17]. Patients who received allo-HSCT in first CR might receive 1 or 2 courses of consolidation chemotherapy before transplantation was performed. The median follow-up time of them was 64 months. Overall survival (OS) was defined as the time period from diagnosis to death or end of follow-up.

2.2. Transplantation

Detailed transplant related characteristics are summarized in Supplementary Table 1. Briefly, 75 patients received peripheral blood stem cell transplantation, 24 patients received bone marrow transplantation, and 1 patient received double-unit cord blood stem cell transplantation. The choices of allo-HSCT for AML patients were made by the responsible attending physicians based on patients' demographic and clinical characteristics. Patients with age <60 years and unfavorable karyotype usually received allo-HSCT in the first CR if HLA-matched donors were available, whereas patients with favorable karyotype did not receive allo-HSCT until relapse. The HLA typing was performed by intermediate-resolution (1995–2007) or sequence-based genotyping methods (since 2007) for loci A, B, C, DR, and DQ. All unrelated donors, except that of the cord blood, came from the Buddhist Tzu-Chi Stem Cell Center in Taiwan. Unrelated donors were either fully matched in six HLA (A, B, and DR) loci (n = 15) or mismatched in one locus (5/6 loci, n = 3). Haploidentical donors (n = 9) have varied HLA matching at 5–9/10 loci.

The choices of conditioning regimens in the allo-HSCT group were also determined by physicians' judgments. Standard busulfan plus cyclophosphamide (BuCy, 48 out of 77 patients) or total body irradiation plus cyclophosphamide (TBICy, 29 out of 77) was used for the myeloablative conditioning (n = 77), whereas reduced intensity conditioning (RIC) with fludarabine 150 mg/m^2 combined with half-dose BuCy was used for the 23 patients with comorbidity. Anti-thymocyte globulin (thymoglobulin, Sanofi, France) 5 mg/kg was given for matched unrelated donor and 6–7.5 mg/kg for mismatched and haploidentical transplantations. Additional fludarabine 90 to 150 mg/m^2 was used in haploidentical transplantations. Cyclosporin-A was used in all patients as the first line graft versus host disease prevention plus either methotrexate in patients receiving myeloablative conditioning (n = 77) or mycophenolate mofetil in patients receiving RIC (n = 23).

2.3. Cytogenetics

Bone marrow cells were harvested directly or after 1 to 3 days of unstimulated culture as previously described [18]. Metaphase chromosomes were banded by the trypsin-Giemsa method and karyotyped according to the International System for Human Cytogenetic Nomenclature. Cytogenetic risk was stratified according to the refined classification of the MRC trial [19].

2.4. Gene mutation

Genetic alterations including *NPM1* [20], *FLT3*-ITD [21], *FLT3*-TKD [22], *CEBPA* [23], *RUNX1* [13], *NRAS* [24], *MLL*-PTD [25], and *WT1* mutations [26] were examined in the recruited AML patients as described previously in the cited references.

2.5. Statistical analysis

All statistical analyses were performed using the SPSS software, version 17 (SPSS Inc., Chicago, IL, U.S.A.) and the XLSTAT software for Microsoft Excel, version 2010.5.02 (Addinsoft, New York, NY, U.S.A.). In statistical testing, two-sided P value $\leq$0.05 was considered statistically significant. The distributional properties of continuous variables were expressed by median and range, categorical variables were presented by frequency and percentage, and the survival curve of OS was estimated by the Kaplan–Meier method. In univariate analysis, the Mann–Whitney U test, chi-square test or Fisher's exact test (if an expected cell frequency of a contingency table <5), and log-rank test were used to examine the differences in the distributions of continuous, categorical, and OS variables between the allo-HSCT and non allo-HSCT groups, respectively. Next, multivariate analysis was conducted by fitting Cox's proportional hazards models to the AML patients in the allo-HSCT and non allo-HSCT groups for identifying important prognostic factors of OS in them.

3. Results

The clinical characteristics and laboratory features for the patients receiving allo-HSCT (n = 100) and those without (n = 225) were shown in Table 1. Except that patients receiving allo-HSCT were younger (35.4 years *vs.* 49.5 years, P < 0.001), there was no difference in other clinical parameters between the two groups. The prevalence of most gene mutations including mutations of *CEBPA* (double or single), *RUNX1*, *NRAS*, *FLT3*-ITD, *FLT3*-TKD, and *MLL*-PTD were similar between allo-HSCT group and non allo-HSCT group (Table 2). However, the prevalence of *NPM1* mutation was lower (13% *vs.* 25%, P = 0.016) and that of *WT1* mutation was higher (13% vs. 6%, P = 0.05) in allo-HSCT group. There was no difference in the distribution of *NPM1*^{mut}/*FLT3*-ITD^{neg} between the two groups. In allo-HSCT group, the source of hematopoietic stem cells came from HLA-identical sibling donors in 73 patients, from unrelated donors in 18, and haploidentical family donors in nine patients. The median time from diagnosis to allo-HSCT was 6.3 months. At