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Original contribution

CD34 expression predicts an adverse outcome in patients with *NPM1*-positive acute myeloid leukemia ☆

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Summary Patients with acute myeloid leukemia (AML) harboring an *NPM1* mutation exhibit a heterogeneous clinical outcome. Recent studies have shown that the absence of *FLT3* internal tandem duplication (*FLT3*-ITD) mutation confers a favorable prognosis in *NPM1*-positive AML. However, the prognostic impact of immunophenotypes in this subgroup remains unclear. In this study, *FLT3* mutation status and immunophenotypic profile of 85 *NPM1*-positive patients with de novo AML were retrospectively analyzed and correlated with their clinical features and survival outcomes. Univariate analysis detected 5 markers with prognostic relevance: older age (≥60 years), high white blood cell (WBC) count (>30 × 10^9/L), *FLT3*-ITD, CD7, and CD34 expression. Multivariate analysis showed that high WBC count was the only independent predictor of a lower complete remission rate (P = .019). Older age (P = .035), high WBC count (P = .008), *FLT3*-ITD (P = .012), and CD34 expression (P = .006) were independent predictors of a shorter event-free survival (EFS). High WBC count (P = .014), *FLT3*-ITD (P = .005), and CD34 expression (P = .047) were independent predictors of a shorter overall survival (OS). Furthermore, based on *FLT3*-ITD status in *NPM1* mutation–positive patients, we showed that both high WBC and CD34 expression conferred a poor EFS (P = .010 and P = .016, respectively) and OS (P = .032 and P = .001, respectively) in the *FLT3*-ITD–negative group, whereas high WBC predicted a poor EFS (P = .016) and OS (P = .027) in the *FLT3*-ITD–positive group. Our results confirm the prognostic value of assessing *FLT3*-ITD mutations in *NPM1*-positive AML and identify the adverse prognostic impact of high WBC and CD34 expression in this subgroup of AML.

1. Introduction

Patients with acute myeloid leukemia (AML) harboring a nucleophosmin 1 (*NPM1*) mutation have distinctive clinicopathologic features and account for approximately 35% of all AML cases [1-3]. Biological features at presentation such as

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immunophenotypes and gene mutations are critical in the prediction of clinical outcome [4]. Moreover, we and others have previously studied molecular genetic and immunophenotypic markers to identify prognostic subgroups for stratification and risk-adapted therapy in AML. Among the markers analyzed, the absence of FMS-like tyrosine kinase receptor-3 internal tandem duplication (*FLT3*-ITD) mutation has been shown to have a favorable prognosis in *NPM1*-positive AML [5-7]. Although immunophenotypes have previously been described in broader contexts in AML, immunophenotypic markers have not been specifically analyzed in previous studies of *NPM1*-positive AML. We and others have identified the surface markers CD7, CD34, CD56, and human leukocyte antigen-DR (HLA-DR) to confer prognostic significance in AML [8-11], but the impact of these immunophenotypes in the context of *FLT3* mutation status among *NPM1*-positive AML has yet to be clarified. We therefore systematically evaluated the prognostic impact of immunophenotypic and molecular genetic markers in a cohort of patients with *NPM1*-positive AML.

2. Materials and methods

2.1. Patients

We screened all patients diagnosed with de novo AML according to the World Health Organization and the French-American-British (FAB) group definition at University Health Network from March 2005 to April 2012. All FAB subtypes except M3 (acute promyelocytic leukemia) were included. In total, 228 patients with AML underwent *NPM1* testing. Among this group, 85 patients tested positive for *NPM1* by multiplex reverse transcriptase polymerase chain reaction (RT-PCR) and comprised our *NPM1*-positive AML cohort for this study. This study was approved by the University Health Network Research and Ethics Board.

2.2. Karyotype analysis

Using standard methods, karyotype analysis was performed on short-term cultures from diagnostic bone marrow specimens. According to International System for Human Cytogenetic Nomenclature (ISCN) guidelines, at least 20 metaphases were analyzed in each case to exclude clonal abnormalities.

2.3. Molecular genetic analysis

Mutation statuses of *NPM1*, *FLT3*-ITD, and FMS-like tyrosine kinase receptor-3 tyrosine kinase domain (*FLT3*-TKD) were evaluated by multiplex RT-PCR [12]. Complementary DNA was obtained via reverse transcription of RNA extracted from bone marrow mononuclear cells. Amplification was performed using primers specific for the *NPM1* and *FLT3*-ITD genes, followed by fragment analysis. *NPM1* and *FLT3*-ITD mutations were identified by size; *FLT3*-ITD mutations were also quantified by determining the percentage of mutant alleles in the sample. The *FLT3*-TKD D835 point mutation was assessed by RT-PCR followed by digestion with the *Eco*RV restriction enzyme [13].

2.4. Immunophenotypic analysis

A whole blood lysis technique was used to process bone marrow or peripheral blood samples [9]. Multiparameter flow cytometric immunophenotyping (EPICSTM XL-MCL; Beckman Coulter, Brea, CA) was performed on leukemic blasts gated on dim CD45 versus low-side scatter and analyzed using combinations of 4 to 5 conjugated antibodies [9]. All samples contained more than 20% blasts with 10 000 list mode events at the blast gate. Antigens expressed by more than 20% of blasts were considered positive.

2.5. Statistical analysis

Biological and clinical factors including age, sex, white blood cell (WBC) count, FAB classification, immunophenotypes, and genetic mutations were evaluated for their influence on 3 outcomes: complete remission (CR) rate, event-free survival (EFS), and overall survival (OS). EFS was measured from treatment initiation until failure to respond, relapse, death, or last follow-up. OS was measured from diagnosis until death or last follow-up.

Fisher's exact test was used to evaluate univariate analysis of the CR rate and correlations between categorical parameters. Univariate analyses of time-to-event outcomes (EFS and OS) were performed using the Kaplan-Meier method and log-rank test. Multivariate analyses for all outcomes were performed including the factors that were significant in univariate analysis for at least 1 of the 4 outcome measures. Only patients who had molecular testing done were analyzed. The Cox proportional hazards model was fitted for EFS and OS. A logistic regression model was fitted for CR rate. Models were reduced using a stepwise procedure removing all variables above the significance level. Statistical tests were performed on SPSS 19.0, and results were interpreted as significant if 2-sided P values were less than .05.

3. Results

3.1. Clinical and laboratory features

There were 40 women and 45 men, with a median age of 56 years (range, 23-74 years) and a median WBC count of 33×10^9/L (range, 0.8-384 $\times 10^9$/L). Of the 85 patients, 82 had normal karyotype belonging to intermediate-risk category, and 3 patients had samples that failed to produce the