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Prognostic factors of patients with newly diagnosed acute promyelocytic leukemia treated with arsenic trioxide-based frontline therapy

Yinjun Lou, Yafang Ma, Shanshan Suo, Wanmao Ni, Yungui Wang, Hanzhang Pan, Hongyan Tong, Wenbin Qian, Haitao Meng, Wenyuan Mai, Jian Huang, Wenjuan Yu, Juyin Wei, Liping Mao, Jie Jin*

Department of Hematology, Institute of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, China

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ABSTRACT

Prognostic factors for patients with acute promyelocytic leukemia (APL) treated in the context of arsenic trioxide (ATO)-based frontline regimes have not been established clearly. We retrospectively analyzed the clinical features, immunophenotypes, Fms-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD), and outcomes of 184 consecutive newly diagnosed APL patients treated by intravenous ATO-based therapy. The median age was 40 years (14–77 years). The early death rate was 4.9% (9/184 patients). With a median follow-up time of 36 months (9–74 months), the 3-year relapse-free survival (RFS) and overall survival (OS) were 93.3% and 92.2%, respectively. Interestingly, there was no meaningful association between 3-year RFS and initial white blood cell count, FLT3-ITD status, or type of PML–RARA isoforms. In multivariable analysis, the CD56 expression was the only independent risk factor in terms of RFS (hazard ratio, 4.70; $P = 0.005$). These results suggested that ATO-based therapy may ameliorate the unfavorable influence of previously known high-risk features; moreover, CD56 expression remains to be a potentially unfavorable prognostic factor in APL patients.

1. Introduction

Although substantial advances have been made in the management of acute promyelocytic leukemia (APL) during the past three decades, treatment failure still occurs in approximately 10–30% of patients because of disease relapse or early death [1–5]. Prognostic factors are essential to realize optimized therapy for APL. A variety of clinical and biological parameters that predict survival in APL have emerged. The Sanz score, which is based on the level of initial white blood cell (WBC) and platelet counts, has been widely accepted as a practical prognostic model for APL patients in terms of risk stratification and treatment selection [6]. The impact of other characteristics, such as body mass index, PML–RARA transcript types, additional chromosome abnormalities, CD56 antigen expression, and Fms-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD), remains unclear.

The Sanz score was established using two well-established cohorts from patients treated with all-trans retinoic acid (ATRA) and idarubicin (AIDA) protocol [6]. The introduction of arsenic trioxide (ATO), which acts synergistically with ATRA to degrade PML–RARA fusion proteins, has significantly changed the treatment paradigm of APL [5,7]. Interestingly, in the past decade several cooperative group trials from across the world have demonstrated the high efficacy of arsenic-containing regimens (mostly intravenous ATO) for frontline therapy in APL [8–16]. In particular, Lo-Coco et al. reported that the chemotherapy-free regimen of ATRA plus ATO may be superior to traditional ATRA plus chemotherapy in the treatment of APL patients with low or intermediate risk [11]. Thus, the addition of ATO-based regimens may be associated with different biologic or clinical prognostic indicators compared with the classic ATRA and chemotherapy protocol. Furthermore, other prognostic factors, such as FLT3-ITD status and CD56 expression, need to be reassessed in this context.

To address these issues, we retrospectively investigated the prognostic relevance of clinicopathological parameters in a well-documented cohort of APL patients treated with ATO-based frontline therapy.

* Corresponding author at: Department of Hematology, Institute of Hematology, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79# Qingchun Road, Hangzhou 310003 China.
E-mail address: zjuhematology@163.com (J. Jin).

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2. Patients and methods

2.1. Patients and therapy

This retrospective, non-interventional, single-center study was conducted at the First Affiliated Hospital Zhejiang University (Hangzhou, China). Pertinent patient clinical reports were obtained with patients' written consent, and approval was obtained from the Ethical Board of the First Affiliated Hospital of Zhejiang University. The study was conducted in accordance to the Declaration of Helsinki and in compliance with institutional guidelines.

The data were collected from consecutive patients with APL who were diagnosed and treated between January 2009 and May 2014 at our institute. The diagnosis of the disease was established on the basis of morphology and immunophenotyping, and confirmed by the presence of *t*(15;17) translocation and/or molecular finding of the specific PML–RARA fusion gene. During the study period, 272 patients were registered in our center. After exclusion (1 refused initial treatment, 2 were transferred to other hospitals, 7 patients died before induction onset, 20 patients were treated with oral tetra-arsenic tetra-sulfide based regimen, and 58 patients were treated with ATRA/chemotherapy regimens), a total of 184 patients under the ATO frontline therapy were recruited in the current analysis. Patients were treated primarily according to the modified Shanghai APL protocol [8,13]. In brief, patients received oral ATRA (25 mg/m^2/day) and intravenous ATO (10 mg/day) daily until documentation of complete remission (CR) for induction therapy, whereas additional minimal chemotherapy was administered to control hyperleukocytosis (idarubicin 6 mg/m^2/day for 2–5 days or daunorubicin 40 mg/m^2/day for 2–5 days or mitoxantrone 1.4 mg/m^2/day for 7–10 days was added if peripheral WBC was greater than 10×10^9/L or on the second day in patients with high risk). After achieving CR, patients were administered 3 courses of conventional chemotherapy consolidation consisting of HA (homoharringtonine 4 mg/m^2/day for 3 days, cytarabine 100 mg/m^2/day for 7 days), MA (mitoxantrone 6–8 mg/m^2/day for 3 days, cytarabine 100 mg/m^2/day for 7 days), and DA (daunorubicin 40–50 mg/m^2 for 3 days, cytarabine 100 mg/m^2/day for 7 days or idarubicin 8–10 mg/m^2 for 3 days without cytarabine). Patients aged >60 years received attenuated chemotherapy cycles. Patients in molecular CR received 5–8 cycles of maintenance treatment with intermittent ATRA and ATO. Aggressive supportive care was provided in accordance to the institutional and Chinese guidelines for APL [17]. Intrathecal therapy prophylaxis was performed 3–4 times after CR.

2.2. Flow cytometry analysis

Immunophenotypic analysis was carried out using bone marrow samples collected at diagnosis and analyzed by conventional immunofluorescence methods. All samples were processed and analyzed within 24 h. The samples were analyzed on the cytometer FACSCanto Becton Dickinson (BD); a minimum of 10^5 WBCs was collected. Data were analyzed using Cell Quest software (BD). Some or all of the following antibodies were used: CD45, CD13, CD33, HLA-DR, CD34, CD2, CD7, CD15, CD19, CD35, CD64, CD71, CD65, CD117, MPO, and CD56. Antigens expressed by more than 20% of the blasts were defined as positive (CD34 was set at 10%). All antibodies were obtained from BD Biosciences.

2.3. Molecular genetic analysis

Regular quantitative real-time polymerase chain reaction (PCR) of PML–RARA transcripts were analyzed as described previously [18]. Longitudinal molecular analysis was conducted in patients for PML–RARA at 3–6 monthly intervals for 3 years. The screening for FLT3-ITD was performed using PCR amplification of genomic DNA and subsequent fragment analysis by capillary electrophoresis on 3130 genetic analyzer (Applied Biosystems, Foster City, CA) as reported previously [19,20]. In selected cases, the presence of FLT3-ITD mutation was confirmed by directional DNA sequencing [21].

2.4. Definitions and statistical analyses

Early death was empirically defined as death within 30 days of admission. Hematologic CR was defined by normalization of bone-marrow morphology to 5% or fewer blast cells and recovery of platelets and neutrophil counts ($>100 \times 10^9$/L and $>1 \times 10^9$/L, respectively). Hematologic relapse was defined as the reappearance of abnormal blast cells or promyelocytes in bone marrow or peripheral blood, or the development of extramedullary disease. Molecular relapse was considered reversion to a positive PML–RARA status confirmed on serial samples within one month after previously documented negative status. Central nervous system (CNS) relapse was confirmed by lumbar puncture and cytologic examination of cerebrospinal fluid.

Relapse-free survival (RFS) was measured from the date of CR to relapse (either hematologic or molecular), death, or last follow-up. Overall survival (OS) was measured from diagnosis until death or last follow-up. The last follow-up was updated on January 15, 2015. Lost to follow-up (2/184, 1.1% from the total cohort) was censored at the time of occurrence. Categorical variables were compared by means of Fisher's exact test or chi-square test. Continuous variables were compared by means of the Mann–Whitney test. The probabilities of RFS and OS were estimated by the Kaplan–Meier method. Odds ratios for early death rate were determined using logistic regression analysis. Multivariable Cox proportional hazard regression models were constructed by entering covariates with $P < 0.20$ in the univariate analysis. A non-parametric bootstrap was performed to ensure the reliable confidence interval. All the above statistical procedures were performed using the SPSS statistical software package version 20.0 and/or GraphPad Prism 5.0 software. All tests were two-sided and values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 summarizes the characteristics of 184 patients at baseline. The median age was 40 years (14–77 years), with 12 patients (6.5%) older than 60 years. Female patients accounted for 46.2% (85/184). When stratified according to Sanz risk score, there were 121 (65.8%) patients in the low-intermediate risk group and 63 (34.2%) in the high-risk group.

Twenty-six (15.9%) of 164 patients showed CD56 expression. Table 2 compares the characteristics of patients with or without CD56 expression. The CD56 expression was significantly associated with short type of the PML–RARA isoform ($P = 0.008$), CD34 expression ($P < 0.0001$), and CD 2 expression ($P = 0.024$). FLT3-ITD data were available in 59.8% (110/184) patients. Twenty-six (23.6%) of 110 patients had FLT3-ITD positive. Table 3 shows details of the presenting features of the FLT3-ITD positive and FLT3-ITD negative groups. The presence of FLT3-ITD correlated significantly with high WBC ($P = 0.002$), low platelet count ($P = 0.020$), and the short type of PML–RARA isoform ($P < 0.0001$). In addition, the FLT3-ITD – positive subgroup was more likely to be CD34 positive (50.0% vs. 15.6%; $P = 0.001$).

3.2. Prognostic factors on early death

The overall early death was seen in 9 patients (9/184, 4.9%) during induction treatment, and 175 out of 184 (95.1%) patients achieved hematologic CR with a median of 29 days (range, 21–42 days). There were no primary resistant patients, and treatment failures were only due to early death. The causes of early deaths were CNS bleeding ($n = 4$), intrapulmonary hemorrhage ($n = 3$), differentiation syndrome ($n = 1$), and sepsis ($n = 1$). Furthermore, the majority (6/9; 66.7%) of these early deaths occurred within the first 7 days of admission.

We then analyzed the association of prognostic factors with the early death rate. As shown in Table 1, the presenting features were compared between the early death and CR group. Early death was associated with high WBC count ($>10 \times 10^9$/L) ($P = 0.0002$), Sanz risk ($P = 0.0001$) and short PML–RARA isoform ($P = 0.005$) at presentation. However, early death was not statistically associated with FLT3-ITD positive, immunophenotypes (CD34, CD56, CD2), or platelet count at diagnosis.

3.3. Prognostic factors impacting on relapse and survival

For the 175 patients who entered hematologic CR, the median follow-up time was 36 months (9–74 months) in surviving patients. By the beginning of the second consolidation, the expression of PML–RARA was negative in 154/171 (90.0%) evaluated patients. All patients went on to achieve molecular CR prior to start of maintenance therapy. The 3-year RFS and OS were 93.3% and 92.2%, respectively. Five patients died (four of disease relapse), two patients missed follow-up, and 168 patients survived. Thirteen patients experienced a relapse, among which two patients had a molecular relapse and three experienced extramedullary disease involving CNS (single or in combination with bone marrow relapse).

Interestingly, CD56 expression subgroup showed inferior 3-year RFS (78.2% vs. 95.9%, $P = 0.005$) (Fig. 1A). However, the FLT3-ITD

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