Leukemia Research 60 (2017) 109–114

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Evaluation of the European LeukemiaNet recommendations for predicting outcomes of patients with acute myeloid leukemia treated in low- and middle-income countries (LMIC): A Brazilian experience☆

Mariana Tereza de Lira Benicio[a,1], Ana Flávia Tibúrcio Ribeiro[b,c,1], Andre D. Américo[a], Felipe M. Furtado[a], Ana B. Glória[c], Aleide S. Lima[d], Silvana M. Santos[f], Sandra G. Xavier[f], Antonio R. Lucena-Araujo[d], Evandro M. Fagundes[c], Eduardo M. Rego[a,e,*]

[a] Department of Internal Medicine, Medical School of Ribeirao Preto, Ribeirao Preto, Brazil
[b] Postgraduate Program in Pathology, Federal University of Minas Gerais, Belo Horizonte, Brazil
[c] Hematology Unit, Federal University of Minas Gerais, Belo Horizonte, Brazil
[d] Department of Genetics, Federal University of Pernambuco, Recife, Brazil
[e] Center for Cell Based Therapy, University of Sao Paulo, Ribeirao Preto, Brazil
[f] Department of Clinical Pathology, Federal University of Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Keywords:
Leukemia
Acute myeloid leukemia
Developing country
Low- and middle-income country
Chemotherapy

ABSTRACT

Background: Current results regarding treatment outcomes in acute myeloid leukemia (AML) point to significant differences between low- and middle-income countries (LMIC) and high-income countries (HIC). Excluding well-known socioeconomic issues, genetic markers important for prognosis have not been properly incorporated into the clinical practice so far and their usefulness outside of well-controlled clinical trials remain unknown.
Methods: Here, we assessed the clinical significance of the European LeukemiaNet (ELN) recommendations in 196 consecutive patients with AML in a real-life setting. All patients were younger than 60 years of age (49% male) and treated with conventional chemotherapy for induction and consolidation in three Brazilian Institutions that well represent Brazilian geographic and socioeconomic diversity.
Findings: Multivariable analysis showed that ELN recommendations had a slight association with complete remission achievement (odds ratio: 0.74, 95% confidence interval, CI: 0.53-1.01; $P = 0.06$), but were independently associated with poor overall survival (OS) (hazard ratio, HR: 1.3, 95% CI: 1.1-1.54; $P = 0.002$), disease-free survival (DFS) (HR: 1.42, 95% CI: 1.03-1.95; $P = 0.028$) and event-free survival (EFS) (HR: 1.24, 95% CI: 1.06-1.47; $P = 0.007$), considering initial leukocyte counts and age as confounders. ELN recommendations had no impact on cumulative incidence of relapse ($P = 0.09$).
Interpretation: Our results suggest that within the context of LMIC, the prognostic markers recommended by ELN may be useful to predict patient's clinical outcomes; however, the OS, DFS and EFS were shorter than the reported in Europe and US for the respective risk groups.

1. Introduction

The implementation of the International Consortium on Acute Promyelocytic Leukemia has proven that, even working with limited resources, significant improvements can be achieved in terms of quality of care and treatment outcome of patients with acute promyelocytic leukemia treated in low- and middle-income countries (LMIC) [1]. The resulting outcomes showed the feasibility to obtaining better results over historical controls [2], and proved that the gap between LMIC and high-income countries (HIC) may be significantly reduced through improving diagnosis, dissemination of essential clinical management guidelines and exchanging of clinical expertise. Yet, little progress on outcomes of non-acute promyelocytic leukemia patients has been reached so far.

Despite scarce, current results regarding the clinical course for adult patients with acute myeloid leukemia (AML) treated in LMIC [3] point

☆ Presented in abstract form at the 53rd Annual Meeting of the American Society of Hematology, San Diego, CA, December 10th, 2011.

* Corresponding author at: Medical School of Ribeirao Preto and Center for Cell Based Therapy, University of Sao Paulo, Av Bandeirantes 3900, Campus USP, Ribeirao Preto, SP, CEP 14048-900, Brazil.
E-mail address: emrego@hcrp.usp.br (E.M. Rego).

[1] M.T.B. and A.F.R. contributed equally to this study.

http://dx.doi.org/10.1016/j.leukres.2017.07.005
Received 7 March 2017; Received in revised form 24 June 2017; Accepted 23 July 2017
Available online 24 July 2017
0145-2126/

to significant disparities compared to HIC [4–8]. This discrepancy lies in multifactorial reasons, and treatment failure of patients with AML in LMIC is often not attributed to the disease *per se*, but rather to socio-economic factors [9] and public health policies-related issues. It is also important to note that most of the data from LMIC refer to a "real world scenario", which is in sharp contrast to those commonly reported by clinical trials. Particularly in Brazil, currently classified as a LMIC, treatment of patients with AML is substantially hampered by the delayed start of induction chemotherapy (because of delayed diagnosis), drug unavailability, and lack of adequate infrastructure for chemotherapy and/or stem cell transplantation. To further complicate this scenario, standard cytogenetic and molecular analyses are still not routinely performed in most Brazilian institutions specialized in hematological malignancies treatment, and translating the current recommendations into improved outcome remains challenging.

To date, the prognostic importance of most schemes for AML risk stratifications have mainly been tested in clinical trials [7,8,10–13], which means that their applicability to determine treatment outcomes for patients with AML outside these well-controlled studies remain unknown. To address this question, we conducted the present analyses by assigning consecutive patients (excluding acute promyelocytic leukemia) from three Brazilian University Hospitals to the proposed genetic groups from the European LeukemiaNet (ELN) recommendations [10] and evaluated their clinical importance in a real-life setting.

2. MATERIALS and METHODS

2.1. Patients' demographics and treatment protocol

Between October 2001 to April 2016, 265 patients with newly diagnosed AML were included. All AML FAB subtypes were considered eligible for study, apart from patients with acute promyelocytic leukemia (FAB M3). Eighty-nine patients were enrolled in Ribeirao Preto (Sao Paulo, southeast Brazil, 34%), while 82 (31%) and 94 (35%) patients were enrolled in Belo Horizonte (Minas Gerais, southeast Brazil) and Recife (Pernambuco, northeast Brazil), respectively. Of note, these cities are representative of the Brazilian diversity in terms of healthcare accessibility and resources. Those differences were further taken into consideration for survival analyses.

All patients were treated with conventional chemotherapy, consisting of daunorubicin (60 mg/m^2 daily for 3 days) and cytarabine (100 mg/m^2 daily for 7 days) as induction therapy, followed by two or three cycles of consolidation therapy with high doses cytarabine ($>$ 1 g/m^2 every 12 h with 3 h infusion on days 1–6). Patients treated in Ribeirao Preto and Recife received 1.5 g/m^2 of cytarabine every 12 h on days 1, 3 and 5, while patients treated in Belo Horizonte received 3 g/m^2 of cytarabine every 12 h on days 1, 3 and 5. For patients who did not achieve complete remission (CR) after one course of chemotherapy, a second course was administered between days 28 and 35 after the end of the first course. CR was assessed by bone marrow examination on day 28 after each course of chemotherapy. Post-remission therapy based on allogeneic transplantation was performed according to the availability of donors and at the discretion of the assisting medical team. The Research Ethics Board of each participating center approved the study. In compliance with the institutional review board-approved protocol (#7147/2005), only excess bone marrow (BM) or peripheral blood collected for diagnostic purpose was analyzed. According to the Declaration of Helsinki, informed consent was obtained from all patients or their relatives.

2.2. Cytogenetic and molecular characterization

All materials used for genetic analyses were obtained at diagnosis and were processed in the reference laboratories of each participating center. Cytogenetic analyses were performed on BM aspirates according to standard techniques for chromosomal banding. For molecular analyses, BM was used whenever available. In all other cases, peripheral blood samples were examined, if samples contained a blast cell count higher than 80%. Standard polymerase chain reaction techniques (and whenever applicable, standard sequencing techniques) were performed for the detection of *FLT3*-internal tandem duplication (ITD), *NPM1* and *CEBPA* mutations [14–17]. For those whose complete data was available, the ELN recommendations [10] for AML risk stratification were used for assigning patients to their respective genetic risk groups.

2.3. Statistics analysis and clinical endpoints

Descriptive analyses were performed for patient baseline features. Fisher's exact test or Chi-square test, as appropriate, was used to compare categorical variables. Kruskal-Wallis test was used to compare continuous variables. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were estimated using the Kaplan–Meier method. OS was defined as the time from diagnosis to death from any cause; those alive or lost to follow-up were censored at the date last known alive. Early mortality was defined as death occurring within 30 days from diagnosis. For patients who achieved CR, DFS was defined as the time from CR achievement to the first adverse event: relapse, development of secondary malignancy, or death from any cause, whichever occurred first. EFS was defined as the time from the initiation of induction therapy to disease relapse, development of secondary malignancy, or death from any cause, whichever occurred first. Patients who were alive without disease relapse or secondary malignancy were censored at the time they were last seen alive and disease-free. The log-rank test was used for comparisons of Kaplan–Meier curves. Cumulative incidence curves for non-relapse death and relapse with or without death were constructed to reflect time to relapse and time to non-relapse death as competing risks [18]. Time to relapse and time to non-relapse death were measured from the date of CR.

Univariable and multivariable logistic regression analyses were performed in order to identify prognostic factors for CR. Univariable and multivariable proportional hazards regression analyses were performed for potential prognostic factors for OS, DFS, and EFS. Potential prognostic factors examined and included in multivariable regression analysis were cytogenetic risk stratification [10], age at diagnosis (analysed as continuous variable), and initial leukocyte counts (analysed as continuous variable). Proportional hazards (pH) assumption for each continuous variable of interest was tested. Linearity assumption for all continuous variables was examined in logistic and pH models using restricted cubic spline estimates of the relationship between the continuous variable and log relative hazard/risk. All *P*-values were two sided with a significance level of 0.05. All calculations were performed using Stata Statistic/Data Analysis version 12 (Stata Corporation, USA), and R 3.3.2 (The CRAN project, www.r-project.org) software.

3. Results

3.1. Baseline features and cohort characterization

Complete data for cytogenetic risk stratification were available for 196 of 265 patients. The baseline features are summarized in Table 1. The median age was 40 years (range: 18–60 years) with 95 males (48%). Overall, 84/196 patients (43%) were cytogenetically normal. *NPM1* and *FLT3*-ITD screening mutations were available for 183/196 (93%) and 185/196 (94%) patients, respectively. Thirty-one patients (17%) had *NPM1* mutations, while forty-five patients (24%) harbored *FLT3*-ITD mutations. *CEBPA* mutations were identified in nine patients (5%). According to the ELN recommendations, patients were stratified as follows: favorable (71 patients; 27%), intermediate I (66 patients; 25%), intermediate II (34 patients; 13%), and adverse (25 patients; 10%). Sixty-nine patients (26%) were not otherwise classified because the G-banding cytogenetics was unsuccessful or complete molecular