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DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

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To the editor:

Clinical outcomes of patients with acute myeloid leukemia: evaluation of genetic and molecular findings in a real-life setting

Despite its undeniable importance, standard cytogenetic analysis is still not a routine practice for most reference centers in developing countries, and its accomplishment is practically limited to university research centers. Particularly in Brazil, little is known about the cytogenetic and molecular characterization of patients with acute myeloid leukemia (AML), and results about clinical outcomes remain scarce. In an effort to minimize the lack of results and contribute new insights in our setting, we reported clinical features and outcomes of 241 nonselected Brazilian patients with AML (nonacute promyelocytic leukemia) from 2 university hospitals, followed from November 2005 to April 2015.

Patients were classified according morphologic, immunophenotypic, and cytogenetic findings. Treatment protocols were similar between centers. For patients up to 60 years of age, the treatment protocol was adapted according to performance status and the presence of comorbidities (in particular, cardiac disorders). Briefly, the conventional chemotherapy consisted of daunorubicin (45-90 mg/m² per day for 3 days) and cytarabine (100-200 mg/m² per day for 7 days) or thioguanine, cytosine arabinoside, and daunorubicin as induction,¹ followed by 3 or 4 cycles of consolidation therapy with high doses of cytarabine (>1 g/m² per day). For patients who did not achieve complete remission (CR) after 1 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. For those who needed it, a postremission therapy based on autologous or allogeneic transplantation was performed. Patients older than 60 years were treated with low-dose cytarabine; a combination of etoposide, thioguanine, and idarubicin; or best supportive care. The local research ethics board

of each participating center approved the study. Research was conducted in accordance with the Declaration of Helsinki.

The baseline characteristics are summarized in supplemental Table 1. One hundred thirty patients were enrolled in Recife (northeast Brazil, 54%), and 111 patients were enrolled in Campinas (southeast Brazil, 46%). Baseline features were similar between centers. The median age was 47 years (range, 18-97 years) with 114 males (47%). Sixty-two patients (26%) were older than 60 years. Pretreatment bone marrow samples were analyzed by G-banding cytogenetics, of which 187 (78%) were successful. According to Medical Research Council trials,² patients were stratified as follows: favorable (30/187, 16%), intermediate (119/187, 64%), and adverse (38/187, 20%). Overall, 101 patients (42%) were cytogenetically normal. To test whether the samples without cytogenetics results were missing at random, the overall survival (OS) was evaluated for patients with and without cytogenetics data. The 5-year OS rate did not differ between patients with (18%) and without (23%) available cytogenetics data ($P = .372$). Additionally, the entire cohort was fully characterized for *NPM1* and *FLT3-ITD* mutations. Details can be found in the supplemental Data, available on the *Blood* Web site.

Out of 241 enrolled patients, 39 patients (16%) who started the induction treatment were lost to follow-up without assessment for CR. Of 202 evaluable patients, 115 (57%) achieved complete hematologic remission. CR rates according to the cytogenetic risk stratification were 33%, 64%, and 77% for adverse, intermediate, and favorable groups, respectively ($P = .001$). The logistic regression analysis revealed that age (odds ratio [OR], 0.95; 95% confidence interval [CI], 0.93-0.98; $P = .002$) and cytogenetic risk stratification (OR, 0.41; 95% CI, 0.19-0.89; $P = .024$) were independently

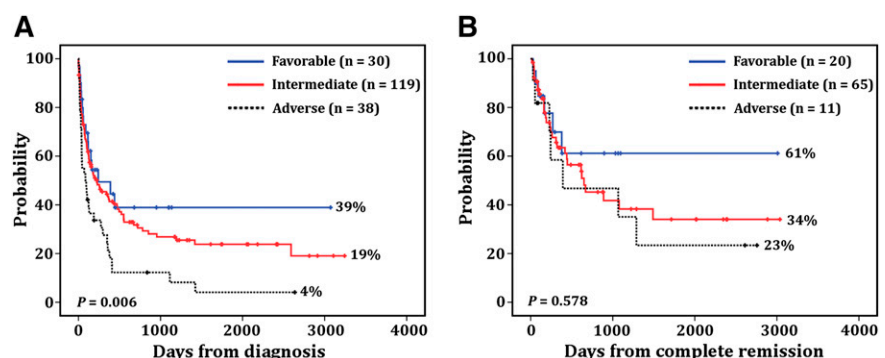


Figure 1. Patient survival. Probability of OS (A) and DFS (B) in patients with AML according to cytogenetic risk stratification.¹ The percentages of each group are presented on the graph. The “n” indicates the number of patients included in each analysis.

associated with CR. The median follow-up among survivors was 177 days (95% CI, 85-268 days). According to Medical Research Council criteria, patients assigned to the adverse group exhibited significantly shorter survival (85 days; 95% CI, 43-169 days) than patients assigned to the intermediate (224 days; 95% CI, 72-375 days) and favorable groups (241 days; 95% CI, 168-572 days) ($P = .006$; Figure 1A). Age (hazard ratio [HR], 1.1; 95% CI, 1.01-1.2; $P = .029$), cytogenetic risk stratification (HR, 1.45; 95% CI, 1.02-2.06; $P = .036$), and white blood cell counts (HR, 1.03; 95% CI, 1-1.06; $P = .021$) were independently associated with poor OS. The median disease-free survival (DFS) for adverse, intermediate, and favorable groups was 392 days (95% CI, 37-1544 days), 650 days (95% CI, 208-1091 days), and 668 days (95% CI, 57-1278 days), respectively. In contrast to OS, cytogenetic risk stratification had no impact on DFS ($P = .578$; Figure 1B).

In summary, we identified patients with favorable, intermediate, and adverse outcomes with frequencies very similar to those reported by other groups.^{2,3} However, our patients' clinical outcomes were significantly inferior to those reported by developed countries. The most significant difference was observed in the favorable group, whose survival rate was significantly lower (39%) than rates reported by studies conducted in the United States and Europe.^{2,4} Furthermore, the early mortality rate was especially higher in our cohort (42%), which is probably a major cause of lower OS rate. Among the main reasons for high early mortality in Brazil, we highlight the lack of adequate hospital infrastructure, especially during induction therapy.^{5,6} As a consequence, high incidence of bacterial and fungal infections are frequently reported.⁷ It is important to note that our results are representative of a real-life setting, which strongly differs from the well-controlled clinical trials conducted in developing countries.

Altogether, our results and others^{1,5,6,8} draw attention to an urgent need for improved clinical support and treatment of patients with AML in order to obtain results comparable to those reported in developed countries. Such improvements may be achieved through international collaborative efforts, which have already proved their effectiveness in economically less privileged countries. A great example is the International Consortium on Acute Promyelocytic Leukemia study.⁹ This initiative considerably reduced the differences in treatment outcome of patients with acute promyelocytic leukemia between developed and developing countries through the dissemination of knowledge and exchange of experience from well-established cooperative groups in the United States and Europe. We hope that with better hospital infrastructure and such initiatives, the clinical outcomes of our patients will improve in the near future.

Aleide S. Lima*

*Department of Genetics, Federal University of Pernambuco,
Recife, Brazil*

Mariana R. de Mello*

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Eliana Fernandes

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Matheus F. Bezerra

*Department of Genetics, Federal University of Pernambuco,
Recife, Brazil
Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

Mayara M. Oliveira

*Department of Genetics, Federal University of Pernambuco,
Recife, Brazil*

Bruno K. Duarte

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Reijane A. de Assis

*Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

Fernanda R. Souto

*Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

Clarisa F. Ramos

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Cintia G. Machado

*Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

Katia Pagnano

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Antonio R. Lucena-Araujo

*Department of Genetics, Federal University of Pernambuco,
Recife, Brazil
Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

Irene Lorand-Metze

*Department of Internal Medicine, Hematology and Hemotherapy Center,
University of Campinas,
Campinas, Brazil*

Marcos A. Bezerra

*Department of Genetics, Federal University of Pernambuco,
Recife, Brazil
Department of Internal Medicine,
Hematology and Hemotherapy Foundation of Pernambuco,
Recife, Brazil*

*A.S.L. and M.R.d.M. contributed equally to this study.

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Correspondence: Antonio R. Lucena-Araujo, Department of Genetics, Center of Biological Sciences, Federal University of Pernambuco, Av Prof Moraes Rego, 1235, Recife, PE 50670-901, Brazil; e-mail: araujoarl@hotmail.com.

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To the editor:

GM-CSF stimulates granulopoiesis in a congenital neutropenia patient with loss-of-function biallelic heterozygous *CSF3R* mutations

Severe congenital neutropenia (CN) is a heterogeneous disease characterized by an absolute neutrophil count (ANC) below 500 cells per microliter and recurrent, life-threatening bacterial infections. Treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF) was shown to increase ANCs in the majority of patients and dramatically improves the quality of life.¹ In a previous study, we demonstrated major differences in responses to G-CSF and granulocyte macrophage CSF (GM-CSF) in patients with CN.² In the majority of these patients, GM-CSF failed to induce neutrophil granulocyte counts but did induce monocytosis and eosinophilia.² Many underlying genetic defects have been identified. Among them are mutations in the *ELANE*, *HAX1*, and *G6PC3* genes and many others.³ A recent study identified patients with biallelic loss-of-function *CSF3R* mutations who were considered to have a novel subtype of CN.⁴ These patients did not respond to G-CSF therapy, and no treatment options were available for them. Heterozygous acquired mutations in the *CSF3R* gene were also reported in CN patients.⁵

In this study, we identified a CN patient who did not respond to G-CSF treatment. Three days after birth, the female patient was diagnosed with CN with blood ANC below 250 cells per microliter. White blood cell differential counts were as follows: 6% neutrophils, 7% monocytes, 4% eosinophils, and 83% lymphocytes. A bone marrow (BM) evaluation at 3 weeks of age revealed granulopoietic hypoplasia with reduction of all stages but no maturation arrest or increase in blasts. Erythropoiesis, lymphopoiesis, and megakaryopoiesis were normal. No antineutrophil antibodies were detected. Mutational screening revealed no mutations in *ELANE*, *HAX1*, or *G6PC3*. Cytogenetic evaluation revealed a normal karyotype.

Sequencing of the *CSF3R* gene showed 2 heterozygous mutations in this patient that revealed a compound heterozygous mode of inheritance of *CSF3R* mutations. In one allele intronic mutation, c.998-2A>T leads to the skipping of exon 9 and introduces an aberrant sequence downstream of exon 8 and a shift in the reading frame. In the second allele, we detected a stop-codon (p.W547*) mutation in the extracellular part of the G-CSF receptor

(G-CSFR) (Figure 1A). The p.W547* mutation was inherited from the father and the c.998-2A>T mutation was inherited from the mother.

No expression of G-CSFR was detected on the patient's neutrophils or monocytes in contrast to blood cells from the healthy donors (Figure 1B). Granulocyte macrophage CSFR (GM-CSFR) expression on CD33⁺ cells from the patient's BM was similar to that observed for BM cells from healthy donors. In addition, we plated the patient's BM mononuclear cells in a semisolid medium supplemented with 10 ng/mL G-CSF, 10 ng/mL GM-CSF, or a cytokine cocktail containing G-CSF, GM-CSF, interleukin-3, stem cell factor, and erythropoietin, and cultured them for 14 days. No granulocyte colony-forming unit (CFU-G), granulocyte macrophage CFU, or macrophage CFU colonies were found in plates supplemented with 10 ng/mL rhG-CSF. In sharp contrast, all types of colonies grew on plates containing either rhGM-CSF or the cytokine cocktail.

G-CSF treatment of this patient was initiated at the age of 3 weeks, but ANCs failed to increase with doses up to 110 µg/kg per day. At the age of 7 months, treatment with GM-CSF (6 µg/kg per day) was initiated. In the first year of GM-CSF treatment, peripheral blood ANCs ranged from 860 to 3744 cells per microliter, enabling the GM-CSF dose to be reduced to 3 µg/kg per day twice a week (Figure 1C). To evaluate whether GM-CSF was still required to maintain sufficient ANCs, GM-CSF administration was stopped with prophylactic administration of oral antibiotics. Because of the development of otitis media, GM-CSF treatment was restarted at a dose of 3 µg/kg twice a week. This dose was sufficient to maintain the patient's ANC above 1000 cells per microliter and kept her free from infections. The patient has remained on GM-CSF treatment for the last 12 years without any adverse events.

In summary, we provide the first demonstration of the successful treatment of a CN patient harboring biallelic loss-of-function *CSF3R* mutations who did not respond to G-CSF by administering GM-CSF. These findings suggest that all CN patients who do not respond to G-CSF should be screened for germ-line *CSF3R*