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Molecular Characterization of Pediatric Acute Myeloid Leukemia: Results of a Multicentric Study in Brazil

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Background and Aims. The biological characterization of childhood acute myeloid leukemia (c-AML) is an important outcome predictor. In Brazil, very little is known about the frequency of AML subgroups, although c-AML accounts for about 18% of leukemias. We carried out this study to investigate the contribution of type I and II gene mutations in the probability of overall survival (pOS) of c-AML in Brazil.

Methods. Seven hundred and three *de novo* pediatric AML cases (2000–2015) were assessed throughout a multicentric network study. Mutations in hotspot regions of *FLT3*, *NRAS*, *KRAS*, *PTPN11*, and c-*KIT* genes were analyzed as well as fusion genes (*RUNX1-RUNX1T1*, *MLL/KMT2A*-r, *CBFβ-MYH11*, and *PML-RARα*) associated with AML. Patients were treated out of the clinical trial although following the BFM-AML2004 protocol. Acute promyelocytic leukemia (APL) was treated differently. AML with Down syndrome was excluded.

Results. There were significant differences in gene mutations among age ranges (≤2 years-old; >2–10 years old and ≥11 years old) and the nonrandom association between type I/II mutations. Lower white blood cell count ($\leq 50 \times 10^9$/L) was associated with *RUNX1-RUNX1T1*, whereas higher WBC with *CBFβ-MYH11* ($p < 0.05$). Cumulative pOS in 5 years was 37.7 ± 2.8% for total AMLs and 59.8 ± 6.2% for APL ($p = 0.03$). pOS differences were observed between Brazilian regions. The South-Southeast regions had a better 5-year pOS, whereas the Midwest region presented the poorest pOS (23.7 ± 4.9%). *PTPN11* mutations conferred an adverse prognosis as an independent prognostic factor.

Conclusions. Identification of genetic subgroups contributes to the molecular epidemiology and biology of AML worldwide, reflecting the profile of pediatric AML cases in Brazil. © 2016 IMSS. Published by Elsevier Inc.

Key Words: Pediatric AML, Brazilian AML, Type I/II mutations, Molecular markers, Prognosis.

Introduction

The improvement of childhood acute myeloid leukemia (c-AML) characterization represents an important challenge in pediatric hematology. In Brazil, little is known regarding the epidemiology and the distribution of biological markers of c-AML, a disease that accounts for 18–24% of all diagnosed cases ≤19 years of age (1). Over the past decade, considerable knowledge has been achieved

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about the leukemia pathogenesis throughout genetic subtypes. Some founder leukemogenic effects are largely described as somatic translocations and fusion genes (2). These recurrent genetic aberrations are important prognostic factors in c-AML and an increasing number of study groups are using them for risk group stratification (3−5). Within the World Health Organization (WHO) classification, these disease-defining genetic aberrations have been used to define unique disease categories and update the new associations over time (6,7).

The leukemogenic process, like another human carcinogenesis pathway, is a consequence of more than one mutation that leads to advantage survival of a single clone and to the heterogeneity of the disease. The two major types of genetic events in AML are type I and II aberrations that, in general, enhance the self-renewal and proliferation potential of the myeloid progenitor. Type I aberrations occur as mutations in hotspots of specific genes involved in signal transduction pathways (*FLT3*, c-*KIT*, *NRAS*, *KRAS* and *PTPN11*), which lead to uncontrolled proliferation and/or survival of leukemic cells. Type II aberrations are often chromosomal rearrangements of transcription factors resulting in the translation of fusion proteins leading to impaired differentiation of the leukemic cells (*PML-RARα* *t*(15,17)(q22;q21), *RUNX1-RUNX1T1* *t*(8,21)(q22;q22), *CBFβ-MYH11* inv(16)(p13q22)/*t*(16,16)(p13;q22) and 11q23/*MLL*(now named as *KMT2A* gene)-rearrangements (8). The third class of aberrations called type III mutations is proposed for AML pathogenesis affecting epigenetic regulators with special reference in adulthood AML (9,10), which reinforces the fact that pediatric cases are a different entity. Moreover, it has been shown that the broad range of type II alterations seems to originate during early life as the first event for overt leukemia (11−13).

A comprehensive analysis of the main type I and II mutations in c-AML was performed, providing an overview of the largest AML series in Brazil as recommended by the WHO for classification of myeloid neoplasms. We determined the distribution frequencies of AML subtypes according to somatic alterations and investigated the potential contribution of these markers with the clinical outcome.

Materials and Methods

Patients

This is a retrospective and multicentric study of 703 *de novo* c-AML received at the Pediatric Hematology-Oncology Research Program (PHOP), Instituto Nacional de Câncer, Rio de Janeiro between January 1, 2000 and December 31, 2015. Cases included were forwarded from 49 Brazilian medical institutions that are reference in oncological care for children with leukemia for diagnostic purpose. The equipped centers were located in four out of five geographical regions of the country (Northeast, South, Southeast, and Midwest) as shown in Figure 1; c-AML was diagnosed according to morphology and immunophenotyping characterization in bone marrow (BM) and peripheral blood (PB) samples from patients aged ≤21 years. Molecular analysis was based on results of the good quality of the diagnostic material. Exclusion criteria consisted of prior chemotherapy, prior myelodysplasic syndrome and associated genetic syndromes (e.g., Down, Bloom, Noonan).

For each center, the information regarding the number of cases was presented as a proportional scale. In order to portray a number of cases in a broad map (Figure 1). Each center performed a review of the morphology according to the WHO/French-American-British (FAB) classification; in six institutions immunophenotyping was also performed and samples were sent to us for additional tests. Clinical and biological data including cytogenetic results when performed were obtained from these medical centers.

A predefined set of data was collected for each case containing clinical data obtained at initial diagnosis, including sex, age, race, and white blood cell (WBC) count. For race, cases were categorized into "whites" and "non-whites" as determined by the parents or guardians of the children. In order to cluster fewer heterogeneity groups. Even though, racial categorization in Brazil is a poor predictor of genomic ancestry (14).

Ethics

The institutional review board approved the treatment according to local laws and guidelines, with informed consent obtained in accordance with the Declaration of Helsinki. For this research project, local agreements and approvals were obtained by the Instituto Nacional de Câncer Research and Ethics Committee under the registry number 186688 as "Immuno-molecular alterations in pediatric AML: an

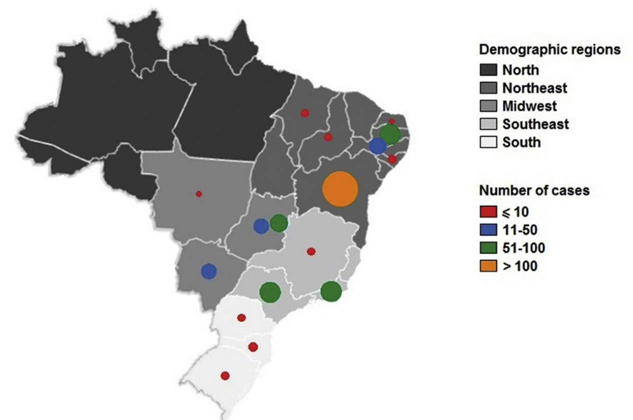

Figure 1. Brazilian map of c-AML cases sent from each geographic region. The number of cases from each collaborative institution is grouped by states and presented as proportional circles. (A color figure can be found in the online version of this article.)

interaction between somatic mutations and etiopathological risk factors''.

Treatment of AML Patients

Patients were treated out of a unique controlled clinical trial but received relatively homogeneous treatment following international consensus guidelines on AML treatment. Due to differences in diagnostic procedures over time, the analyses of treatment and prognostic markers with relevance were divided into two periods: cases diagnosed from 2000–2007 (first period) and those from 2008–2015 (second period). Treatment strategies were mainly standardized with two different induction regimens using cytarabine, idarubicin, and etoposide as the BFM-AML2004 protocol in the second period of the case ascertainments. The clinical decisions in the majority of settings followed the guidelines on the treatment of AML published elsewhere (15). Stem cell transplantation was performed in selected high-risk patients (16). Acute promyelocytic leukemia (APL) cases were treated with all-trans retinoic acid and specific therapeutic strategies (17).

Identification of Type I Mutations

Genomic DNA was purified from BM samples with QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Mutations were determined in the hotspot regions of *FLT3*, *NRAS*, *KRAS*, *PTPN11*, and c-*KIT* as previously described or following primers and PCR conditions provided in Supplementary Table 1 (18). Briefly, *FLT3* mutations were examined at the tyrosine kinase domain (TKD) in codon 835 and juxtamembrane domain in exons 11/12 as internal tandem duplications (ITD). *NRAS*/*KRAS* status was determined by searching mutations in exon 1 (with special attention in codons 12/13), *PTPN11* mutations were screening in exon 3, and c-*KIT* mutations were identified in exons 8/17.

Identification of Type II Mutations

Total RNA was purified from BM cells using TRIzol® reagent (Invitrogen, Carlsbad, CA). cDNA was synthesized following transcriptase reverse enzyme addition and cDNA integrity was evaluated throughout *GAPDH* amplification (19). Type II mutations were screened for the four most frequent fusion genes in pediatric AML cases including *RUNX1-RUNX1T1*, *CBFβ-MYH11*, *KMT2A*-rearrangements (*KMT2A*-r) and *PML-RARα*. The last one was directly performed according to acute promyelocytic leukemia (APL) morphology and immunophenotyping profile. The fusion genes and *KMT2A* breaks were also identified by fluorescence *in situ* hybridization (FISH) with commercial DNA probes (PML/RARα Translocation Probe Dual Fusion; AML1/ETO Translocation Probe Dual Fusion; CBFβ/MYH11 Translocation Probe Dual Fusion; MLL Dual Color Break Apart Rearrangement Probe; Cytocell, UK).

The fusion transcripts were confirmed as described elsewhere (19,20). The most common fusion partners of *KMT2A* gene (*MLLT3/AF9*, *MLLT10/AF10*, *MLLT1/ENL*, *MLLT4/AF6*, *AFF1/AF4*) were identified by reverse transcriptase polymerase chain reaction according to Burmeister et al. (20).

Statistical Analysis

Descriptive analyses were performed through continuous variables in order to measure central tendency and dispersion, as well as categorical variables to determine the frequency distribution. χ^2 or Fisher's exact test was used to compare proportions between groups. The non-parametric Mann-Whitney *U* test was used for continuous variables. For the purpose of this study, age was considered a categorical variable with three groups for analysis, comprising cases who were ≤2 years old, between 2 and 10 years old and ≥11 years old. Also, the distribution of cases was tested for distinct periods: first period (2000–2007) and second period (2008–2015) of c-AML diagnosis.

Overall survival (OS) was measured from the date of diagnosis to the date of the last follow-up or death from any cause. Patients who did not experience an event were censored at the time of the last follow-up and those who did not attend a follow-up were censored at their date of last known contact. Kaplan-Meier survival analysis method was used to calculate the 5-year probabilities of OS (pOS), and estimated survival values were compared using the log-rank test in order to verify the association of the presence of one genetic alteration in patients' outcome. Association between independent variables and outcome was performed using Cox proportional-hazard regression model with estimated hazard ratio (HR) and 95% confidence intervals (CI) were presented. Variables with $p < 0.20$ were included in this model for multivariate analysis following the stepwise forward method. All p values were considered significant when <0.05. All analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, 2004).

Results

Demography and frequency of c-AML subtypes according to period of incident cases are described in Table 1. There was a high frequency of cases from the Northeast region ($p < 0.001$) and an increased number of c-AML from the Midwest region was observed in the second period of case ascertainments. There were no differences regarding age groups and race distributions over time and the majority of c-AML were considered as non-white (61.2%). The overall age distribution of patients showed a median age of 7.3 years, ranging from 0–21 years. Cases aged ≤2 years old represented 24.0%, those with ages between 2 and 10 years represented 37.7% and those patients aged

Table 1. Demographic characteristics and classification of pediatric AML cases according to periods of analysis, Brazil, 2000−2015

AML features	First period (2000−2007), *n* (%)	Second period (2008−2015), *n* (%)	*p*
Brazilian geographic regions			<0.001
Northeast	179 (59.1)	170 (42.5)	
South	14 (4.6)	15 (3.8)	
Southeast	78 (25.7)	98 (24.5)	
Midwest	32 (10.6)	117 (29.2)	
Age groups (years)			0.32
≤2	81 (26.7)	88 (22.0)	
>2−10	108 (35.6)	157 (39.2)	
≥11	114 (37.6)	155 (38.8)	
Race			0.31
Whites	86 (36.3)	161 (40.4)	
Non-whites	151 (63.7)	238 (59.6)	
Sex			0.01
Males	183 (60.4)	203 (50.8)	
Females	120 (39.6)	197 (49.2)	
ICD-O3 code			0.004
9806/3 (Mixed phenotype acute leukemia with *BCR-ABL1*)	1 (0.3)	2 (0.5)	
9809/3 (Mixed phenotype acute leukemia, T/myeloid)	1 (0.3)	3 (0.8)	
9840/3 (Acute erythroid leukemia)	12 (4.0)	5 (1.2)	
9861/3 (Not otherwise specified, NOS)	21 (6.9)	14 (3.5)	
9866/3 (Acute promyelocytic leukemia; *PML-RARα*)	49 (16.2)	81 (20.2)	
9867/3 (Acute myelomonocytic leukemia)	32 (10.6)	65 (16.2)	
9871/3 (Acute myeloid leukemia, *CBFβ-MYH11*; FAB M4Eo)	8 (2.6)	28 (7.0)	
9872/3 (AML with minimal differentiation)	14 (4.6)	14 (3.5)	
9873/3 (AML without maturation)	18 (5.9)	17 (4.2)	
9874/3 (AML with maturation)	31 (10.2)	21 (5.2)	
9891/3 (Acute monoblastic/monocytic leukemia)	25 (8.3)	41 (10.2)	
9896/3 (Acute myeloid leukemia, *RUNX1-RUNX1T1*)	33 (10.9)	40 (10.0)	
9897/3 (11q23 abnormalities)	33 (10.9)	41 (10.2)	
9910/3 (Acute megakaryoblastic leukemia)	25 (8.3)	28 (7.0)	
Status[a]			
Alive	123 (40.6)	170 (42.5)	<0.001
Dead	87 (28.7)	165 (41.5)	
Missing	93 (30.7)	65 (16.2)	
Total, *n* (%)	303 (43.1)	400 (56.9)	

FAB, French-American-British classification; ICD-O3 International Classification of Diseases for Oncology 3rd Edition; WHO, World Health Organization.
[a]Excluding acute promyelocytic cases.

≥11 years represented 38.3%; c-AML occurred in different frequencies among boys and girls in the first period of the study, with male predominance ($p = 0.01$), corresponding to a male:female ratio of 1.5:1 in the first period and 1:1 in the second period. Differences among the c-AML subtype classification over time were observed, mainly with a decrease of not otherwise specified (NOS)-AML subtype in the second period of the study.

The frequency distribution of type I and II molecular alterations according to age strata and AML morphology are shown in the supplementary material. The number of type I mutations increased in the upper ranges of age, i.e., cases aged ≤2 years old presented 12.5% of type I mutations, cases ranging from >2−10 years old and ≥11 years old presented 39.5% and 47.7% of type I mutations, respectively, as shown in Supplementary Table 2. Non-white cases presented more type I mutations than cases considered white, with 68.7 and 31.3% ($p = 0.001$), respectively. These differences were not observed for type II mutations. Both type I/II mutations were associated with morphological subtypes, with the majority of them occurring in AML cases with myelomonocytic differentiation. We identified more than one type II mutation occurring concomitantly in 13.2% of the cases.

The distribution frequencies of molecular alterations in c-AML according to demography and WBC count at diagnosis are shown in Table 2. All type II mutations presented an association with age range. *RUNX1-RUNX1T1*, *CBFβ-MYH11* and *PML-RARα* were more frequent in cases aged ≥11 years (48.6, 56.5, 57.1%, respectively). Similarly, both types of *FLT3* mutations (ITD/TKD) were found with older cases (55.8, 66.7%, respectively). c-AML with 11q23/*KMT2A* fusion genes grouped as *KMT2A*-r were found in children at an early age (≤2 years old) in 67.6% of cases compared with AML without *KMT2A*-r in different age groups ($p < 0.001$). The frequency of partner gene was

Table 2. Distribution frequency of molecular alterations in c-AML cases according to demography and white blood cells count, Brazil, 2000–2015

		Age (years)					Sex			WBC count ($\times 10^9$/l)			
Molecular alteration[a]	**Frequency, *n*/total (%)**	**Median (range)**	**≤2, *n* (%)**	**>2–10, *n* (%)**	**≥11, *n* (%)**	***p***	**Males, *n* (%)**	**Females, *n* (%)**	***p***	**Median (range)**	**≤50, *n* (%)**	**>50, *n* (%)**	***p***
Type II mutations													
RUNX1-RUNX1T1	74/390 (12.9)	9.3 (0.2–18.3)	9 (12.2)	29 (39.2)	36 (48.6)	<0.001	44 (59.5)	30 (40.5)	0.29	20.1 (1.7–136)	58 (80.6)	14 (19.4)	0.001
CBFβ-MYH11	23/376 (6.1)	13.3 (0.3–19.3)	4 (17.4)	6 (26.1)	13 (56.5)	0.04	10 (43.5)	13 (56.5)	0.30	111.0 (7.2–268)	6 (27.3)	16 (72.7)	<0.001
KMT2A rearrangements	74/308 (24.0)	1.3 (0.0–21.1)	50 (67.6)	16 (21.6)	8 (10.8)	<0.001	36 (48.6)	38 (51.4)	0.19	54.1 (2.4–451)	36 (50.0)	36 (50.0)	0.004
PML-RARα	63/87[b] (72.4)	11.2 (1.3–18.0)	2 (3.2)	25 (39.7)	36 (57.1)	0.06	31 (49.2)	32 (50.8)	1.00	10.8 (0.1–800)	48 (77.4)	14 (22.6)	0.34
Type I mutations													
FLT3 (ITD or TKD)	110/473 (23.3)	11.0 (1.0–21.3)	5 (4.5)	41 (37.3)	64 (58.2)	<0.001	60 (54.5)	50 (45.5)	0.88	33.5 (0.8–800)	65 (59.1)	45 (40.9)	0.10
FLT3-ITD	86/473 (18.2)	10.9 (1.0–21.3)	3 (3.5)	35 (40.7)	48 (55.8)	<0.001	47 (54.7)	39 (45.3)	0.88	35.6 (0.8–540)	50 (58.1)	36 (41.9)	0.10
FLT3-TKD	24/473 (5.1)	11.9 (1.8–19.3)	2 (8.3)	6 (25.0)	16 (66.7)	0.001	13 (54.2)	11 (45.8)	0.97	25.8 (2.5–800)	15 (62.5)	9 (37.5)	0.61
KRAS	30/464 (6.5)	4.6 (0.5–18.3)	9 (30.0)	12 (40.0)	9 (30.0)	0.73	20 (66.7)	10 (33.3)	0.14	40.4 (1–700)	16 (53.3)	14 (46.7)	0.16
NRAS	44/410 (10.7)	10.2 (0.7–18.0)	8 (18.2)	13 (29.5)	23 (52.3)	0.27	24 (54.5)	20 (45.5)	0.91	48.5 (5.1–800)	23 (52.3)	21 (47.7)	0.07
c-*KIT*	22/210 (10.8)	5.0 (0.3–19.3)	5 (22.7)	12 (54.5)	5 (22.7)	0.29	13 (59.1)	9 (40.9)	0.32	45.2 (4.5–168)	12 (57.1)	9 (42.9)	0.51
PTPN11	27/260 (10.4)	7.5 (0.4–17.1)	5 (18.5)	13 (48.1)	9 (33.3)	0.66	18 (66.7)	3 (20.0)	0.20	40.0 (1.0–300)	17 (63.0)	10 (37.0)	0.53

ITD, internal tandem duplication; TKD, tyrosine kinase domain; WBC, white blood cell count at diagnosis.
[a]The total numbers of analyzed cases reflect the availability of biological material for molecular tests.
[b]The total of *PML-RARα* analyzed was considered among the acute promyelocytic leukemia.

MLLT3 (20/74, 27.0%), *AFF1* (10/74, 13.5%), *MLLT10* (7/74, 9.5%), *MLLT1* (4/74, 5.4%) and *MLLT4* (2/74, 2.7%), and other less frequent such as *KMT2A-PTD* (data not shown in tables). Type II aberration *RUNX1-RUNX1T1* was associated with low WBC count ($\leq 50 \times 10^9$/l, 80.6%), whereas *CBFβ-MYH11* was associated with high WBC count ($>50 \times 10^9$/l, 72.7%). All the association results were confirmed in a WBC count analysis as continuous variable by the Mann-Whitney U test (data not shown). *NRAS* mutations were associated with higher WBC count at diagnosis (median 48.5×10^9/l for *NRAS* mutated cases vs. 29.5 $\times 10^9$/l for wild-type cases; $p = 0.02$). We observed that mutations in *KRAS* and *PTPN11* mutations seem to present a different male:female ratio from the overall cohort, with 2:1 and 6:1, respectively.

Overall distribution frequencies of type I among type II mutations are represented in Figure 2. Association between the presence of any type I mutation and fusion genes in c-AML showed that *RUNX1-RUNX1T1* and *PML-RARα* are the aberrations presenting the higher number of type I mutations (28.6 and 33.8%, respectively; data not shown). The variation frequencies of *FLT3, KRAS* and *NRAS* mutations in all of the type II subtypes were observed, which seem to be differently distributed among them.

Statistical values calculated for the nine molecular alterations, including p values and OR, are shown in Supplementary Table 3. c-*KIT* mutations were more frequently observed within the core-binding factor (CBF)-AML group, including both *CBFβ-MYH11* (OR 3.1, 95% CI 1.1–9.1) and *RUNX1-RUNX1T1* (OR 3.7, 95% CI 1.2–10.9), compared to other type II subtypes.

The univariate analysis for pOS in both periods of ascertainment cases and the potential contribution of the molecular markers in clinical outcome are shown in Table 3. No statistically significant associations were found for age, sex and WBC count in the overall cohort according to the two periods of analysis. The median of pOS time of the whole AML cohort, excluding APL, was 17.1 months (95% CI 12.9–21.2 months; 5-year pOS 37.7 ± 2.8%); for APL, the 5-year pOS was 59.8 ± 6.2% and the median was not reached. In the first period of the study, the variables that presented significant pOS values were age strata, the presence of *FLT3* mutations and APL as shown in Figures 3A–3C.

In the second period of the study, the region where the treatment was placed showed differences in 5-year pOS (shown in Figures 4A–4F). Cases from the South/Southeast region of Brazil presented better outcome (5-year pOS 54.7 ± 6.5%) than others. AML with *CBFβ-MYH11* and APL with *PML-RARα* had better 5-year pOS than other AML subtypes ($p = 0.03$ and $p = 0.08$, respectively). AML with *PTPN11* mutations were associated with the worst 5 year pOS ($p < 0.001$).

Five-year pOS showed large differences between type II aberrations as described as a whole in detail in Table 3. We

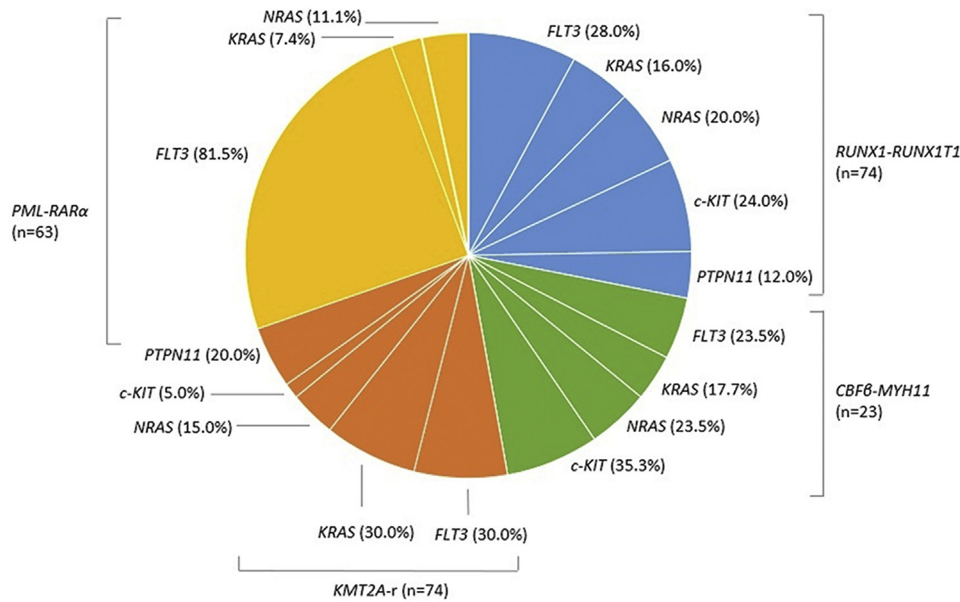

Figure 2. Pie chart illustrating the frequency of type II mutations among subgroups of type I mutations in c-AML. (A color figure can be found in the online version of this article.)

compared positive vs. negative cases for the specific type II mutations except for *PML-RARα*, within which analysis was performed in order to compare cases with *PML-RARα* and other genetic subtypes. Patients carrying the fusion genes *CBFβ-MYH11* or *PML-RARα* showed the most favorable outcome with 5-year pOS of 67.9 ± 10.9% and 72.8 ± 7.0%, respectively, as well as identified by Cox proportional hazard model (Supplementary Table 4). Patients presenting *KMT2A*-r (different fusion genes together) showed the lowest 5-year pOS (35.0 ± 6.9%) compared to cases negative to this molecular alteration.

For type I mutation analysis, patients presenting *NRAS* and c-*KIT* mutations had a better outcome than the other AML cases, with 5-year pOS of 57.8 ± 9.0% and 58.1 ± 12.7%, respectively; however, this was not statistically significant. On the other hand, cases presenting *PTPN11* mutations showed the worst prognosis with 5-year pOS of 10.4 ± 9.0% (Table 3 and Supplementary Table 4). Multivariate analysis identified *PTPN11* mutations as an independent prognostic factor to predict adverse pOS (HR 2.3, 95% CI 1.3−4.1; $p = 0.004$).

Discussion

This retrospective analysis of *de novo* c-AML cases gathered throughout molecular diagnostic characterization represents the largest AML series in Brazilian children and adolescents. The substantial amount of cases was only possible due to the joint effort of collaborating institutions that sent the samples for diagnostic characterization and epidemiological studies (21). These cover ~70% of the main pediatric onco-hematology hospital in the Brazilian regions. Because of the morphological and molecular heterogeneity of AML, our study has made it possible to identify subgroups defined by molecular aberrations, adapting the diagnosis to recommendations of specific treatments and to understand the biology of the disease (5). In addition, the AML classification based on morphological features is not sufficient to predict prognostic value.

The present study demonstrated the high percentage of APL (16.2−20.2%) in both the first and second periods of analysis followed by the AML myelomonocytic subtype (10.6−16.2%) among the entire AML setting. The NOS-AML category in WHO classification is a universal concern because it gathers AML with heterogeneous biology that evolved with different distributions throughout time. NOS-AML frequency was found especially high in the first period of the study (2000−2007 = 6.9%) compared to the second period (2008−2015 = 3.5%) when an algorithm of tests allowed allocating cases in a specific AML subgroup. Considering that more sensitive and specific molecular tests such as FISH were accessed in Brazil over time, APL remains more frequent than other subtypes. APL group still represents the main subtype specified in the Brazilian series in both periods, before and after the inclusion of molecular markers. The present results reinforce the premises and fulfill gaps on AML epidemiology and molecular profile characterized by a high rate of APL/*PML-RARα* in children and younger adults. By that means, this study leads to the great effect of knowledge about c-AML subtypes in a Latin American country. High proportions of APL have been found in regions such as Italy, Spain, Central America and South America (22).

Table 3. Univariate analysis for overall survival parameters of c-AML cases, 2000–2015, Brazil

	n (*n* of events)	Univariate analysis		*p**
		5-year pOS (SE)	Median[a] (95% CI)	
Periods of analysis[c]	443 (218)			0.38
Phase I (2000–2007)	171 (81)	40.1 (4.4)	19.6 (7.3–32.0)	
Phase II (2008–2015)	272 (137)	36.3 (3.7)	15.4 (10.1–21.2)	
Geographic regions of treatment[b]	443 (218)			0.003
Northeast	197 (87)	37.8 (4.8)	18.0 (10.9–25.1)	
South/Southeast	139 (63)	47.6 (4.7)	40.7[e]	
Midwest	107 (68)	23.7 (4.9)	10.5 (3.4–17.7)	
Age range (years)[b]	443 (218)			
≤2	147 (79)	34.6 (4.9)	13.9 (6.6–21.2)	0.40
>2–10	148 (68)	38.8 (2.1)	21.8 (10.5–33.1)	0.14
≥11	148 (71)	38.8 (4.7)	11.8 (5.0–18.7)	0.49
Race[c]	407 (200)			0.29
Whites	168 (84)	40.5 (4.5)	19.6 (6.7–32.5)	
Non-Whites	239 (116)	33.3 (4.1)	15.2 (10.0–20.7)	
Sex[b]	443 (218)			0.93
Males	250 (124)	35.2 (3.9)	19.1 (13.0–22.7)	
Females	193 (94)	40.1 (4.2)	16.3 (10.0–22.7)	
WBC count ($\times 10^9$/l)[b]	428 (211)			0.64
≤50	284 (134)	39.2 (3.5)	17.5 (13.5–21.5)	
>50	144 (77)	34.5 (4.9)	13.8 (5.8–21.8)	
Morphological classification	545 (252)			0.02
Acute promyelocytic leukemia	102 (34)	59.8 (6.2)	[d]	
Other subtypes	443 (218)	37.7 (2.8)	17.1 (12.9–21.2)	
Type II mutations[c]				
RUNX1-RUNX1T1[b]	329 (170)	36.9 (7.9)	16.6 (9.5–23.7)	0.53
CBFβ-MYH11[b]	318 (168)	67.9 (10.9)	[d]	0.02
KMT2A rearrangements[b]	260 (131)	35.0 (6.9)	10.3 (3.3–17.3)	0.25
PML-RARα[d]	545 (252)	72.8 (7.0)	[d]	0.003
Type I mutations[bc]				
FLT3	388 (183)	26.1 (6.7)	13.5 (7.4–19.6)	0.01
KRAS	383 (176)	46.8 (10.9)	47.4[e]	0.60
NRAS	334 (159)	57.8 (9.0)	[d]	0.13
c-*KIT*	186 (80)	58.1 (12.7)	[d]	0.28
PTPN11	206 (93)	10.4 (9.0)	4.3 (0.3–8.4)	0.003
Concomitant type I mutations[b]	131 (68)			0.16
Single mutation	115 (56)	36.9 (5.8)	19.1 (12.2–25.9)	
More than one mutation	16 (12)	25.0 (10.8)	2.4 (0.0–10.9)	

CI, confidence interval; *n*, number; pOS, probability of overall survival; SE, standard error.
**p* values from log-rank test indicate whether the differences are significant between subgroups.
[a]Median survival in months.
[b]Excluding acute promyelocytic leukemia subtype.
[c]Analysis performed between groups positive and negative for the molecular alteration.
[d]Median not reached.
[e]Confidence intervals undefined.

The present findings have an impact on enhancing epidemiological studies involving genetic markers and requiring environmental exposures investigations as well as to guide strategies for tailoring treatment. APL subgroup is at the same time highly fatal and highly curable, deserving a separate and urgent treatment strategy (23,24).

Comparing the present data with the recent publication of AML with hospital-based registries and a population-based study, the findings confirm the differences in regional distribution of c-AML. For instance, we have noticed a male predominance noted in all c-AML groups found here, which corroborates with findings from hospital-based and population-based cancer registries (25,26). Moreover, different host factors such as sex, race, body mass index and genetic background have been associated with increased risk of AML development (27). The findings that mutations in *KRAS* and *PTPN11* were more frequent in males supports the premises that both genes would be involved in the proliferation and survival of spermatogonial stem cells (28).

All subtypes of AML probably share abnormalities in common pathways that regulate proliferation, differentiation, and cell death. These include mutations that affect proliferative and cell survival signals, and mutations that

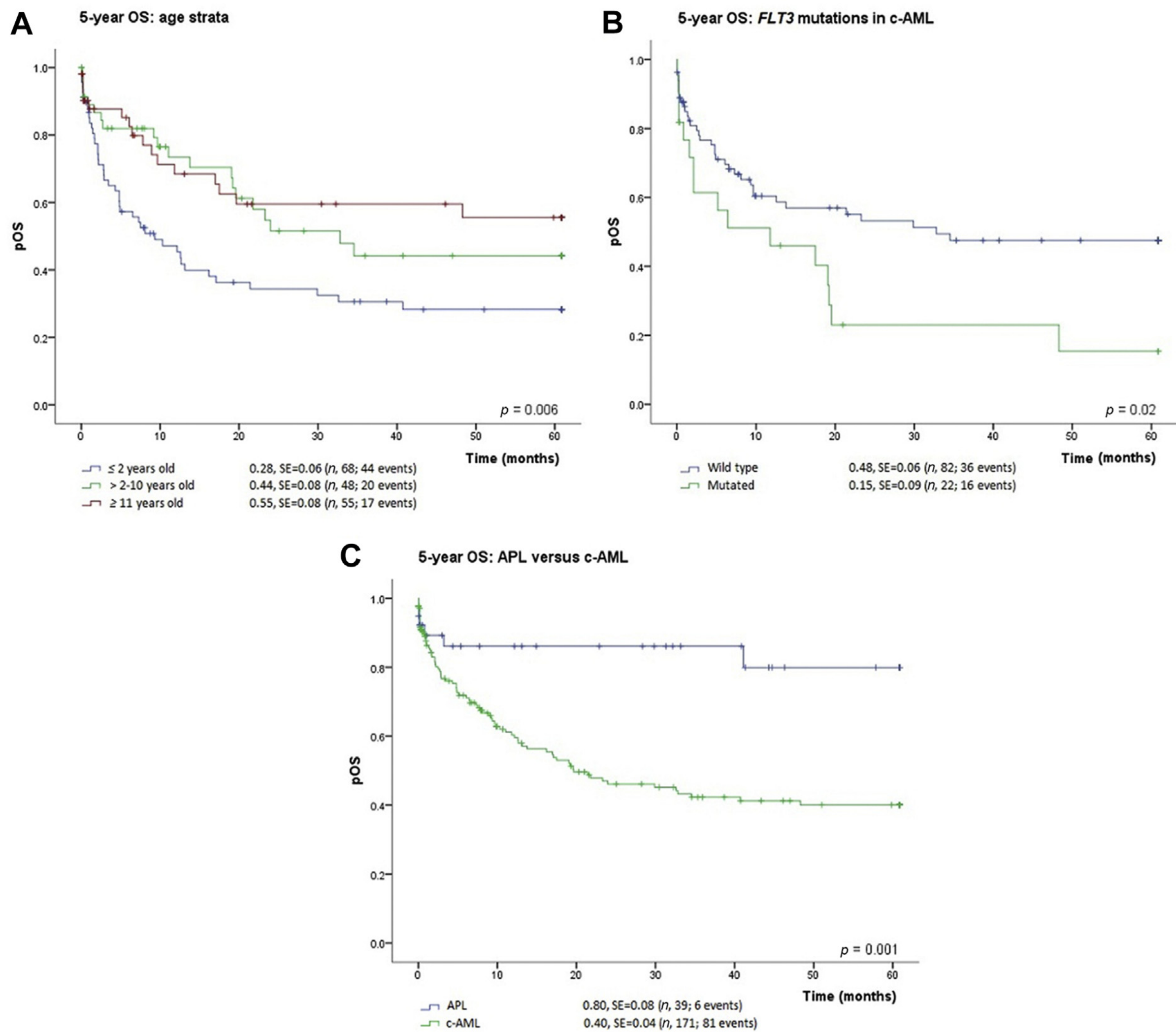

Figure 3. Survival analysis of the c-AML cases of the First Period (2000−2007). Kaplan-Meier estimates for the probability of overall survival (pOS) for age strata (A), *FLT3* mutations in c-AML (B), and APL (C). *p* values were calculated using log-rank test. SE, standard error. (A color figure can be found in the online version of this article.)

lead to differentiation arrest or enhanced cell self-renewal (8). A discussion about the value of molecular markers in the c-AML treatment should begin with recognizing that various prognostic subtypes are closely linked to the chromosomal karyotypes present in the leukemia cells as the founder element of AML pathogenesis (29). The karyotype and/or molecular aberration allow the segregation of c-AML into three categories of favorable, adverse, and intermediate prognosis subgroups. Our results corroborate with the literature as *CBFβ-MYH11* and *PML-RARα* are predictors of favorable outcomes. The prognostic significance of other genetic abnormalities individually could not be assessed because of the small number of cases in each subgroup.

AML with *RUNX1-RUNX1T1* and *CBFβ-MYH11* have been recognized as unique entities among AML with their predictive values and both aberrations affect CBF and are usually named and gathered as CBF-AMLs. However, considerable clinical and biological heterogeneity exists within CBF-AMLs (30). For instance, we found that *RUNX1-RUNX1T1* was associated with low WBC count, whereas *CBFβ-MYH11* was associated with high WBC count. The pathogenic mechanisms of *CBFβ-MYH11* responsible for high proliferation rate and consequently high WBC count provide an effective benefit evaluation. Therefore, high WBC count in subsets of AML should be considered with caution and molecular markers become more relevant tools than WBC count at diagnosis. Distinct molecular events that result in leukocytosis might be markers inherent of the disease, underscoring the pathogenic consequences of both rearrangements.

The distribution of type II aberrations in infant AML (i-AML) differs in older patients as *RUNX1-RUNX1T1* and *PML-RARα* are seldom found in these settings,

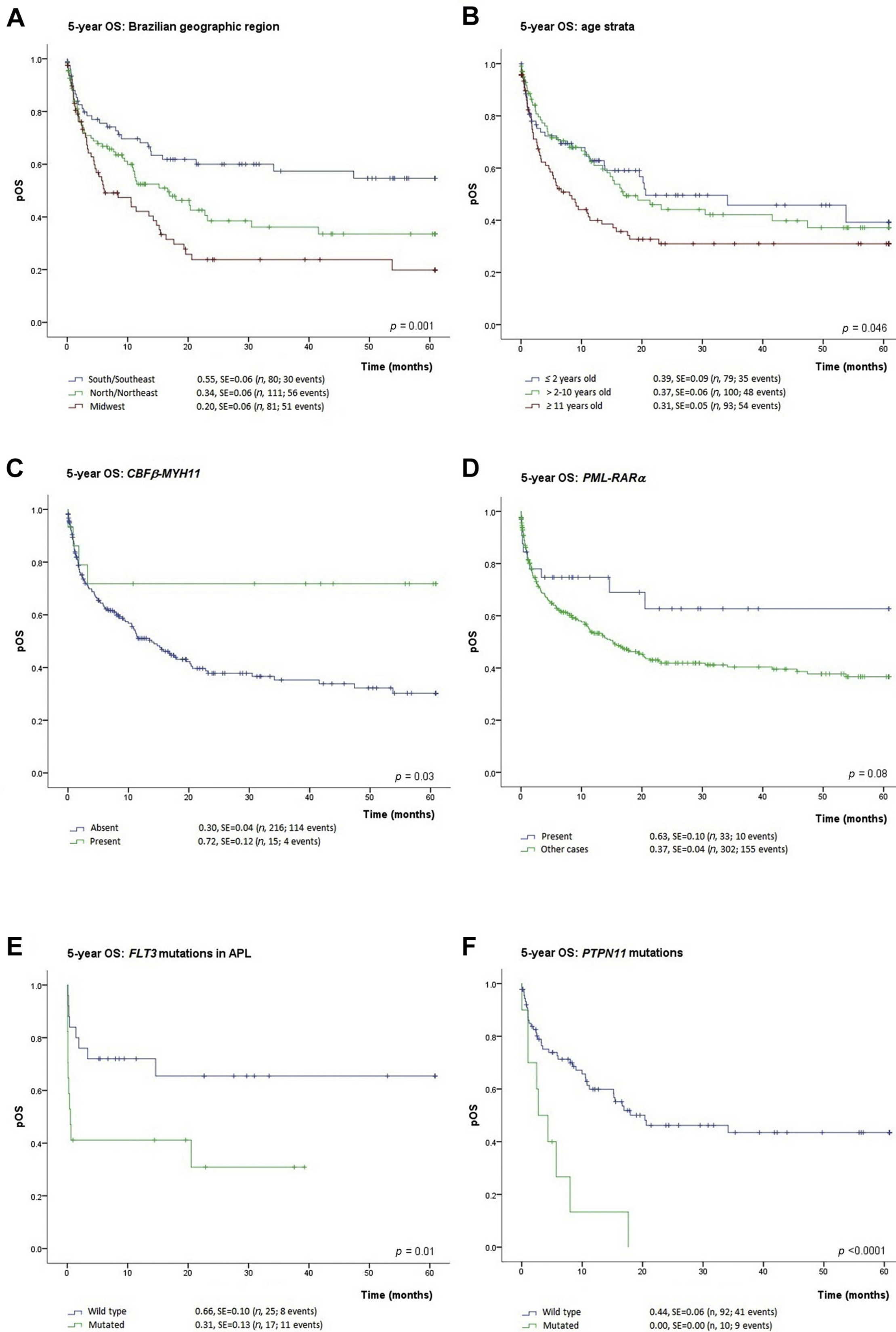

Figure 4. Survival analysis of the c-AML cases of the second period (2008–2015). Kaplan-Meier estimates for the probability of overall survival (pOS) for geographic region (A), age strata (B), presence of *CBFβ-MYH11* (C), presence of *PML-RARα* vs. other cases (D), *FLT3* mutations in APL (E), and *PTPN11* mutations in c-AML (F). *p* values were calculated using log-rank test. SE, standard error. (A color figure can be found in the online version of this article.)

suggesting different mechanisms of leukemogenesis. As a whole, our findings are similar to those reported previously (31). Nevertheless, we found a higher frequency proportion of i-AML with associated with *KMT2A*-r (67.6%) compared to that described in the literature (31,32). A possible explanation for this phenomenon could be the fact that we are a reference for infant leukemia diagnosis (33). However, these findings deserve further ecological investigation. Concerning the prognostic predictive values, i-AML have been associated with adverse prognostic features (34). Additionally, in this current study, patients aged ≥11 years presented an inferior outcome as demonstrated previously (35), pointing out biological parameters in different age groups of c-AML patients. As outcome data strongly depend on treatment, it is important to evaluate whether different age groups have been treated similarly. There is also a big difference for children and adolescents to access treatment in clinical trials, which might influence prognosis (35−37). Survival data for the specific c-AML are rarely found in the medical literature.

Regarding the findings of type I mutations (5,7), we identified similar frequencies in *FLT3*, *NRAS* and c-*KIT* mutations compared to what has been described in adulthood AML, although slightly lower frequencies were identified than described in c-AML elsewhere (31,38−41). The recurrent somatic mutations *FLT3* and *PTPN11* may allow additional refinement in prognostication and, in some cases, would provide opportunities for targeted treatment.

Limitations of these data should be acknowledged and considered in order to interpret the findings. First of all, the results are based on analysis of retrospective data collected in 49 treatment centers, which might have caused constraints on adherence to returns and lack of information requested. The lower death rate (28.7%) and better pOS in the first period of the study would affect the differences in the estimate rates due to the higher proportion of missing information on follow-up (30.7%) when compared with the second period of the study (16.2% of missing reports). One speculation is that in the first period, although with a low number of centers with adherence to this project, they had more controlled management therapeutic protocols, whereas in the second period of the study the increased number of centers located in less developed regions and low research resources compromised the overall pOS. Another pitfall is concerning the variable (mode of treatments) that catalyzes the improvements in AML. They were not assessed in this study (as it is in controlled therapeutic protocol) such as AML risk-stratification and management support care.

Even with these limitations, it is noteworthy to explain that patient access to the national public health system (SUS created in 1988) reorganized and allowing free treatments, and the access to PHOP research facilities to leukemia characterization, the molecular and epidemiological scenarios described herein, certainly reflect the reality of Brazil over time (21). The high number of patients included and the wide time frame covered enabled us to have a representative frequency of distribution of c-AML. Although the clinicians and the infrastructure of each center involved in AML treatment were off-clinical trial research, a variety of guideline procedures such as supportive care and management of complications and information and comparison with published results were shared through dissemination of innovation care in the network (24,42). Nevertheless, additional clinical controlled studies would be necessary to reinforce the present results.

In conclusion, differences in OS ratio were observed between Brazilian regions, with the South-Southeast regions with better 5-year pOS, whereas the Northeast-Midwest regions had the poorest OS. Although they are not population-based, these findings contribute with important data in the epidemiology scenario of c-AML as a whole. Survival data for the specific c-AML subtype in Latin American countries are rarely found in the literature. Inclusion of cytogenetic-molecular markers in the characterization of AML are of great predictive value for OS. *PTPN11* mutations conferred adverse prognosis as an independent prognostic factor.

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Supplementary Data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.arcmed.2016.11.015.

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