MOLECULAR CHARACTERIZATION OF PEDIATRIC ACUTE MYELOID LEUKEMIA

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BACKGROUND

❖ In Brazil, little is known about the epidemiology and the distribution of biological markers of childhood acute myeloid leukemia (c-AML), a disease that accounts for 18-24% of all diagnosed cases <19 years of age.

❖ Recurrent translocations and fusion genes are important prognostic factors in c-AML and has been used for risk group stratification (Table 1).

Table 1. Genetically defined prognostic groups in pediatric AML

Prognosis	Genetics
Favorable	t(8;21)(q22;q22)/*RUNX1-RUNX1T1*
	inv(16)(p13.1q22)/*CBFb-MYH11*
	t(15;17)(q22;q21)/*PML-RARa*
Intermediate	t(9;11)(p22;q23)/*KMT2A-MLLT3*
	t(1;22)(p13;q13)/*RBM15-MKL1*
Poor	t(6;9)(p23;q34)/*DEK-NUP214*
	t(4;11)(q21;q23)/*MLL-MLLT2(AF4)*
	t(10;11)(p12;q23)/*MLL-MLLT10(AF10)*
	t(6;11)(q27;q23)/*MLL-MLLT4(AF6)*
	t(5;11)(q35;p15.5)/*NUP98-NSD1*
	t(9;22)(q34;q11.2)/*BCR-ABL1*

Creutzig et al., Blood, 2012

❖ Rare genetic alterations, including *DEK-NUP214*, *NUP98* rearrangements (*NUP98*-r), *RBM15-MKL1, MYST3-CREBBP* and *CBFA2T3-GLIS2* demand to be investigated to better understand the biology of the disease.

❖ Somatic mutations in genes known to regulate hematopoiesis, as *RAS* pathway genes, have been identified in c-AML, and the presence of these mutations has been shown to be associated with leukemogenesis, as well as, clinical outcome.

❖ Our aim was to identify the more frequent molecular alterations in c-AML cases, including recurrent fusion genes and RAS pathway affected genes and to investigate the potential contribution of these findings in the estimated probability of overall survival (pOS).

MATERIAL AND METHODS

Patients. This is a retrospective and multicentric study of 785 de novo c-AML referred to the Pediatric Hematology-Oncology Research Program, INCA, Rio de Janeiro, between January 1, 2000 and May 31, 2017. Cases included were forwarded from 49 Brazilian medical institutions that are reference in oncological care for children with leukemia for diagnostic purpose (Figure 1).

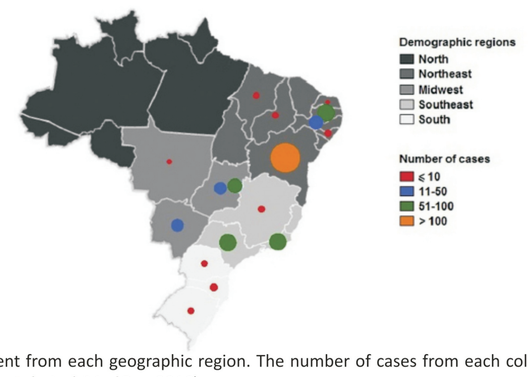

Figure 1. Brazilian map of c-AML cases sent from each geographic region. The number of cases from each collaborative institution is grouped by states and presented as proportional circles (Andrade et al., Arch Med Res, 2016).

Treatment. Patients were treated out of a unique controlled clinical trial but received relatively homogeneous treatment following international consensus guidelines on AML treatment, with two different induction regimens using cytarabine, idarubicin, and etoposide as the BFM-AML2004 protocol after the year 2008.

Molecular alterations. Mutations in hotspot regions of *RAS* pathway affecting genes *(FLT3, NRAS, KRAS, PTPN11,* and *KIT*) were analyzed by direct sequencing. 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 (codons 12/13), *PTPN11* mutations were screening in exon 3, and *KIT* mutations were identified in exons 8/17.

Fusion genes associated with c-AML were screened by RT-PCR and/or FISH [*MLL/KMT2A* rearrangements *(KMT2A-r*), *RUNX1-RUNX1T1, CBFb-MYH11, PML-RARa, NUP98-r, CBFA2T3-GLIS2, MYST3-CREBBP,* and *RBM15-MKL1*].

Statistical Analysis. Statistical analysis was performed taking into account descriptive frequencies of variables in order to measure central tendency and/or dispersion. Fisher's test was applied to compare proportions between subgroups. Mann-Whitney U test was used for continuous variables. Age strata were considered a categorical variable as three groups: ≤ 2 years-old; >2-10 years old and >11 years old. The Kaplan-Meier survival analysis was used to calculate the 5 year pOS and estimated survival values were compared using the log-rank test. Cox proportional-hazard regression model with estimated hazard ratio (HR) and 95% confidence intervals (CI) were presented.

RESULTS

Table 2. Frequency of molecular alterations in c-AML cases according to demography and white blood cells count, Brazil, 2000-2017

		Age (years)					Sex			WBC count (x10⁹/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
Fusion genes[b]													
RUNX1-RUNX1T1	80/547 (14.6)	9.3 (0.1-20.5)	9 (5.7)	33 (18.1)	38 (19.1)	<0.001	48 (16.9)	32 (12.5)	0.16	21 (1.7-136)	62 (18.0)	16 (6.8)	0.003
CBFβ-MYH11	29/532 (5.5)	10.7 (0.3-19.3)	5 (3.2)	9 (5.2)	15 (7.7)	0.18	12 (4.3)	17 (6.9)	0.21	106 (2.2-373)	8 (2.4)	20 (11.1)	<0.001
KMT2A-r	46/497 (9.3)	1.6 (0.0-21)	55 (34.0)	23 (14.7)	12 (7.2)	<0.001	45 (17.3)	45 (20.1)	0.43	44.4 (1.0-451)	49 (15.1)	39 (25.7)	0.006
PML-RARα[c]	109/137 (80.2)	10.8 (1.3-19)	2 (66.7)	50 (76.9)	57 (82.6)	0.61	62 (80.5)	47 (78.3)	0.75	10.6 (15.8-228)	79 (79.0)	29 (80.6)	0.84
NUP98-r	12/70 (17.1)	1.7 (0.8-13.9)	7 (30.4)	3 (13.6)	2 (10.5)	0.19	5 (17.2)	7 (20.0)	0.78	36.9 (5.9-318.7)	7 (14.9)	5 (29.4)	0.19
MYST3-CREBBP[d]	3/22 (13.6)	1.0 (0.0-1.8)	na	na	na	na	1 (7.7)	22 (22.2)	0.33	8.6 (5.7-111)	2 (13.3)	1 (14.3)	0.95
CBFA2T3-GLIS2	1/62 (1.6)	na	1 (2.0)	0 (0.0)	0 (0.0)	0.89	1 (3.3)	0 (0.0)	0.30	na	1 (2.2)	0 (0.0)	0.57
RBM15-MKL1[e]	2/37 (5.4)	0.5 (0.2-0.7)	2 (11.1)	0 (0.0)	0 (0.0)	0.33	2 (11.8)	0 (0.0)	0.12	14 (7.2-20.8)	2 (6.7)	0 (0.0)	0.55
RAS pathway mutations													
FLT3 (ITD or TKD)	125/544 (23.0)	11.1 (1.0-21.0)	5 (4.2)	46 (22.1)	73 (34.4)	<0.001	67 (23.0)	57 (22.9)	0.97	34.7 (0.8-800)	72 (20.7)	52 (28.4)	0.046
KRAS	34/535 (6.4)	6.0 (0.5-18.3)	10 (7.9)	13 (6.5)	11 (5.4)	0.66	21 (7.4)	13 (5.3)	0.32	33.6 (1.0-700)	19 (5.6)	15 (8.2)	0.25
NRAS	49/479 (10.2)	10.2 (0.7-18.0)	8 (8.7)	15 (8.2)	26 (13.0)	0.26	27 (10.3)	22 (10.3)	0.98	50 (5.1-800)	25 (8.3)	24 (14.4)	0.04
KIT	23/262 (8.8)	5.4 (0.3-19.3)	5 (6.9)	12 (11.7)	6 (7.1)	0.44	13 (10.2)	10 (7.6)	0.46	42.7 (4.5-168)	13 (7.8)	81 (90.0)	0.55
PTPN11	34/372 (9.1)	8.2 (0.4-17.1)	5 (7.2)	15 (9.9)	14 (9.4)	0.81	23 (11.4)	11 (6.5)	0.11	40.2 (1.0-374)	21 (8.8)	13 (10.5)	0.59
Outcome (%)													
5y-pOS (SE)[f]	469/613 (76.5)	na	33 (4.8)	43.1 (4.5)	43.7 (4.1)	0.10	39.2 (3.6)	42.2 (3.7)	0.73	na	44.6 (3.2)	32.2 (4.3)	0.003

[a] The total number of analyzed cases reflect the availability of biological material for molecular tests. [b] *DEK-NUP214* was not found in any of the 59 cases screened. [c] *PML-RARα* analysis was considered versus the remaining cases. [d] *MYST3-CREBBP* was analyzed among cases aged ≤2 years old with hemophagocytosis. [e] *RBM15-MKL1* was analyzed among cases of acute megakaryoblastic leukemia. [f] Excluding acute promyelocytic leukemia. ITD, internal tandem duplication; na, not applicable; TKD, tyrosine kinase domain; WBC, white blood cell count at diagnosis; 5y-pOS, estimated probability of overall survival in 5 years.

Table 3. Association between fusion genes and *RAS* pathway mutations in c-AML

Molecular alteration[a,b]	*KMT2A-r* n pos (%)	*RUNX1-RUNX1T1* n pos (%)	*CBFβ-MYH11* n pos (%)	*PML-RARα*[c] n pos (%)	*NUP98-r* n pos (%)	*MYST3-CREBBP*[d] n pos (%)	*CBFA2T3-GLIS2* n pos (%)	*RBM15-MKL1*[e] n pos (%)
RAS pathway mutations								
FLT3 (ITD or TKD)	**7 (9.7)**	**7 (10.4)**	5 (17.2)	37 (50.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
KRAS	8 (10.8)	4 (6.3)	4 (14.3)	2 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
NRAS	3 (5.7)	6 (10.7)	4 (14.8)	4 (5.9)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
KIT	1 (2.3)	**6 (25.0)**	**6 (23.1)**	1 (3.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
PTPN11	7 (14.6)	3 (6.8)	0 (0.0)	**1 (1.6)**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a] The total number of analyzed cases reflect the availability of biological material for molecular tests. [b] *DEK-NUP214* was not found in any of the 59 cases screened. [c] *PML-RARα* analysis was considered versus the remaining cases. [d] *MYST3-CREBBP* was analyzed among cases aged ≤2 years old with hemophagocytosis. [e] *RBM15-MKL1* analyzed among cases of acute megakaryoblastic leukemia. ITD, internal tandem duplication; pos; positive; TKD, tyrosine kinase domain. P<0.05 in bold.

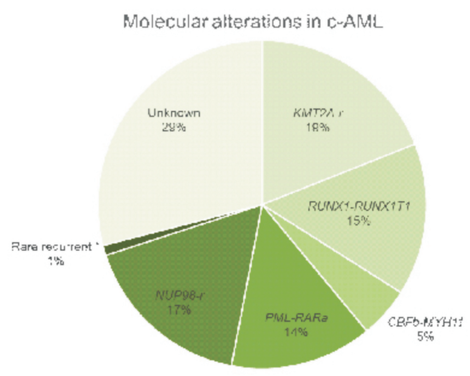

Figure 2. Molecular alterations represented by fusion genes found in childhood AML. Rare recurrent abnormalities include *MYST3-CREBBP*, *CBFAT23-GLIS2* and *RBM15-MKL1* that represent <1% of cases.

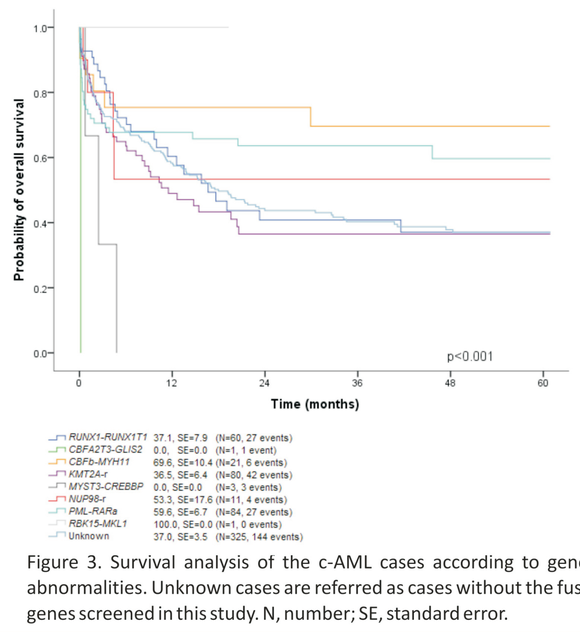

Figure 3. Survival analysis of the c-AML cases according to genetic abnormalities. Unknown cases are referred as cases without the fusion genes screened in this study. N, number; SE, standard error.

CONCLUSION

The identification of genetic subgroups contributes to the molecular epidemiology and biology of AML worldwide, reflecting the profile of pediatric AML cases in Brazil. Survival data for the specific c-AML subtype in Latin countries are found rarely in the literature. The inclusion of cytogenetic-molecular markers in the characterization of AML is of great predictive value for pOS. Diagnosis and management of c-AML raise questions that should be considered for further action.

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