CYTO-MOLECULAR APPROACHES DETECTED NOVEL GENE REARRANGEMENTS AND 16,5% OF COMPLEX KARYOTYPES IN 146 BRAZILIAN CHILDREN WITH ACUTE MYELOID LEUKEMIA: AN ORIGINAL WORK

Roberto Rodrigues Capela de Matos[1], Thomas Liehr[2], kelly Cristina de Assis Monteso[1], Amanda Faria de Figueiredo[1], Mariana Tavares de Souza[1], Gerson Moura Ferreira[1], Renata Binato[1], Marcelo GP Land[3], Raul Correa Ribeiro[4], Maria Luiza Macedo Silva[1]

[1]Instituto Nacional de Câncer – INCA; [2]Jena University Hospital; [3]Instituto de Puericultura e Pediatria Martagão Gesteira – IPPMG; [4]St. Jude Children's Research Hospital

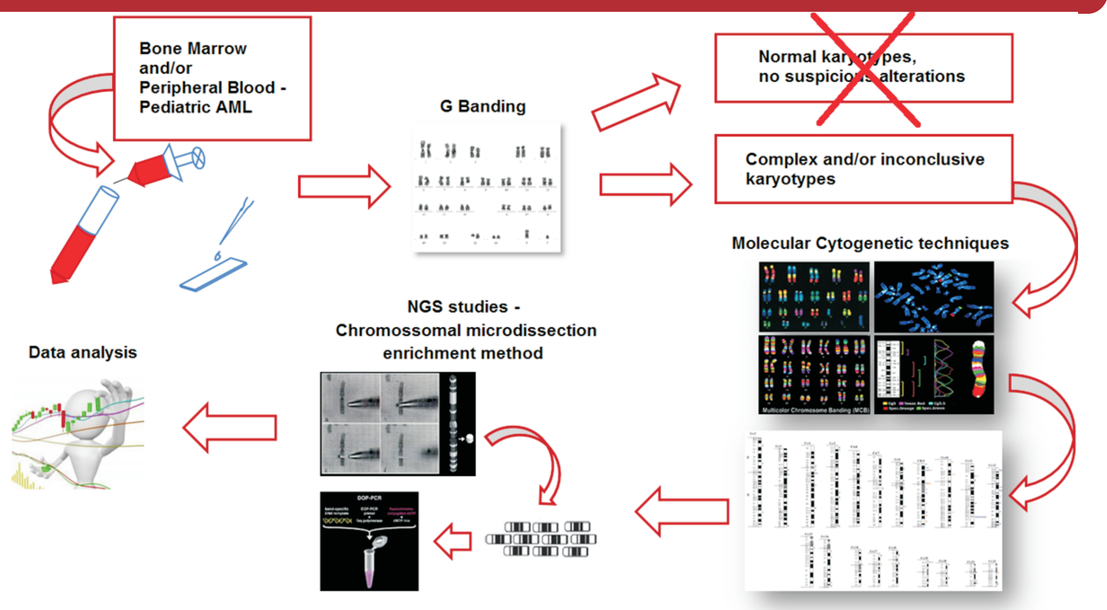
Objectives

Acute Myeloid Leukemia (AML) represents a heterogeneous group of hematologic malignancies. In AML, cytogenetic alterations can be found associated with the risk groups according to the WHO classification and related to morphological subtypes according to the FAB classification FAB. The t(8;21)(q22;q22) / *RUNX1-RUNX1T1*, associated with the FAB M2 subtype, t(15;17) (q22;q21) / PML-RARα, associated with FAB M3, inv(16)(p13;q22) / *CBFβ-MYH11*, associated with M4, which are related to a favorable prognosis, according to the treatment response. On the other hand, patients with -5 or del(5q), -7 or del(7q), inv(3q) / t(3;3)(q21;q26) / *GATA2* / *MECOM*, the t(6;9)(p23;q34) / *DEK-NUP214*, del(9q), t(9;22)(q34;q11) / *BCR-ABL1, KMT2A* gene rearrangements, and complex karyotypes, with these abnormalities and/or other cryptic aberrations, are selected for high-risk protocol, including bone marrow transplantation.

The literature presents an incidence of 10% of complex karyotypes in AML, which may cryptically carry one or more chromosomal abnormalities which may be responsible for adverse prognosis. Also, it has been suggested a correlation between the set of aberrations detected in a complex karyotype, and the clinical response, which makes clear the need for refining this karyotype profile.

In this work, we aimed to detect and characterize, through the combination of conventional (G-banding) and molecular (fluorescence in situ hybridization, Multiplex-FISH, and multicolor chromosome banding), complex and/or inconclusive karyotypes, in order to contribute to a better definition of the karyotype profile of pediatric patients diagnosed with AML.

Methods



**This project was approved by the research ethics committee of INCA (#088/07).*

Conventional Cytogenetics

Cytogenetic analysis was performed at diagnosis, before treatment administration, in bone marrow samples cultured for 24 hours according to the standard protocol.

Molecular Assays

Fluorescence *in situ* hybridization-based experiments were conducted using both, homemade and commercial probes, according to the manufacturers' instructions.

** The karyotypes were described according to the International System for Human Cytogenetic Nomenclature.*

Results

Bone marrow and/or peripheral blood samples from 146 pediatric patients diagnosed with AML were sent to the Laboratory of Cytogenetics (CEMO - INCA Specialized Laboratories Division).

13 (8.9%) did not present mitosis. Of the 133 samples presenting mitosis, 11 (8.2%) had normal karyotypes. The percentage of chromosomal abnormalities was 91.8%.

Of the 133 samples that presented mitosis and were refined, 22 (16.5%) were characterized as complex karyotypes.

We observed a ratio of 1:1, with 11 male and 11 female patients, with a median age of 7 years old (min. 6 months and max. 16 years).

The median leukocyte count was 16.950 (min. 2.000 and max. 692.000), and the median platelet count was 73.500 (min. 11.000 and max. 318.000).

A death rate of 41% was observed.

Patient	Morphological Subtype	Treatment Protocol	Status after first induction	Relapse	BMT	Current Clinical status
1	M1	AML-BMF-2004	Remissão completa	Recaída medular isolada, durante a manutenção	Não realizou	Óbito
2	M4	AML-BMF-2004	Remissão completa	Não apresentou	Não realizou	Vivo
3	M5	Perda de seguimento	-	-	-	Óbito
4	M4/M5	AML-BMF-1998	Remissão completa após 2ª indução	Recaída medular isolada, após tto	Não realizou	Óbito
5	M4/M5	AML-BMF-2004	Remissão completa após 2ª indução	Recaída medular isolada, após tto. Atingiu 2ª remissão	TMO aparentado / TMO aparentado	Óbito
6	M4	Perda de seguimento	-	-	-	Óbito
7	M3	AML-BMF-2004	Remissão completa	Não apresentou	Não realizou	Vivo
8	M5	Ensaio clínico AML02	Remissão completa após 2ª indução	Não apresentou	Não realizou	Vivo
9	M1/M2	Perda de seguimento	-	-	-	Óbito
10	M1/M2	AML-BMF-2004	Falha de indução. Remissão completa após 3º bloco de QT	Não apresentou	Não realizou	Óbito
11	M2	AML-BMF-2004	Remissão completa	Não apresentou	Não realizou	Vivo
12	M2	AML-BMF-2004	Remissão completa	Não apresentou	Não realizou	Vivo
13	M1	AML-BMF-2012	Falha de indução. Remissão completa após 3º bloco de QT	Não apresentou	TMO não aparentado	Vivo
14	M4	AML-BMF-2004	Remissão completa	Recaída medular isolada, durante a manutenção	Não realizou	Óbito
15	M1	Não realizou	-	-	Não realizou	Óbito
16	M4/M5	Perda de seguimento	-	-	-	Viva
17	M4	AML-BMF-2012	Remissão completa	Não apresentou	Não realizou	Viva
18	M2	AML-BMF-2004	Remissão completa após 2ª indução	Não apresentou	TMO não aparentado	Viva
19	M5	AML-BMF-2004	Remissão completa	Não apresentou	TMO não aparentado	Viva
20	M3	AML-BMF-2004	Remissão completa	Não apresentou	Não realizou	Vivo
21	M5	AML-BMF-2012	Remissão completa após 2ª indução	Não apresentou	Não realizou	Vivo
22	M2	AML-BMF-2012	Falha de indução. Remissão completa após 4º bloco de QT	Não apresentou	Não realizou	Viva

- 16 patients BFM protocol variants / 1 patient experienced the clinical trial AML02;
- 13 patients (59%) alive / 9 patients (41%) deceased.
- Complete remission at first induction in 9/18 children;
- First indiction failure 8/18;
  - 5 patients achieved complete remission after second induction;
  - 3 patients achieved complete remission at consolidation.
- 4 patients disease relapse;
  - 2 patients relapsed after chemotherapy;
  - 2 patients relapsed during treatment.
- 3/4 patients which experienced BMT had good response to the treatment.

Patient	Morphological subtype	G - Banding	FISH	WCP/PCP/M-FISH/MCB
1	M1	46,XY,del(9)(q31),t(?X;?11)(p22;q22) [11] / 46,XY [9]	NR	46,XY,del(9)(q12;q31),del(11)(q13 or q14) [4] / 46,XY,del(9)(q12;q31) [2] / 46,XY [7]
2	M4	46,XY,inv(16)(p13q22),+19,-22 [18] / 46,XY [4]	NR	46,XY,inv(16)(p13;q22),dic(22)(qter->p10::p10->qter) [6]
3	M5	44,XX,?der(4),-5,-7,-10,?dic(13),-15,+2 mar [18]	NR	45,XX,der(5)t(5;19)(q13.3;q12),t(6;18;17)(q24.3;q21;q23.2),der(7)t(7;13)(p11.2;q12),der(10)(7qter->7q11.23::12q22->12q21.2::10p14->10q21::12q12->12qter),der(12)(::p13->q12::), -15,del(19)(q12),der(20)t(20;21)(p11.2;q11.2),der(21)t(12;21)(q22;q22) [8]
4	M4/M5	46,XY,t(1;11;8)(q21;p21;q23) [25]	r-*KM2TA*	46,XY,der(1)t(1;8)(q21;p21),der(8)t(1;8)(q31;p21),der(11)ins(11;1)(q23;q21q31) [6]
5	M4/M5	45,X,-Y,del(8)(q22),add(22)(q22),add(22)(q22) [22]	*RUNX1-RUNX1T1*	45,X,-Y,t(8;22;21)(q21.3;q13.3;q22.12),der(22)t(1;22)(q23;q13.3) [5]
6	M4	46,XX,del(16)(q2?) [8] / 46,XX [14]	NR	46,XX,inv(16)(p13;q22),del(16)(q22) [14]
7	M3	46,XY,-6,t(15;17)(q22;q21),+mar [21]	*PML-RARα*	46,XY,t(15;17)(q22;q21),inv(6)(p24;q15) [10]
8	M5	46,XY,del(10)(p12) [18] / 46,XY [2]	r-*KM2TA*	46,XY,der(10)(11qter->11q23.3::10q11.2->10p12::10q11.2->10qter),der(11)(11pter->11q23.3::10p12->10p12::11q23.3->11q23.3::10p12->10pter) [6]
9	M1/M2	47,XX,der(2)t(2;?)(q2?;?),t(3;13)(q13;p11),add(5)(q35),t(16;21)(p11;q22) [14]	*RUNX1-RUNX1T1 / CBFβ*	46,XX,der(2),t(2;3)(q2?;?),t(3;13)(q13;p11),der(5)t(3;5)(?;q35),t(16;21)(p11;q22) [7]
10	M1/M2	45,X,-X,del(8q22),+der(13q3?) [23]	*RUNX1-RUNX1T1*	45,X, -X, t(8;13;21)(q22;q33;q22) [6]
11	M2	45,X,-Y,del(2)(q33),t(8;21)(q22;q22),del(9)(q22) [13]	*RUNX1-RUNX1T1*	NR
12	M2	46,X-Y,del(8q22),+der(17q?24) [22]	*RUNX1-RUNX1T1*	45,X-Y,t(8;17;21)(q22;q21;q22) [5]
13	M1	46,XY,der(2)?t(2;15),del(5),der(14)?add(14)(q23) [16]	del *EGR1*	46,XY,t(2;14)(q23.1;q32.2),t(2;15)(p22.3;q21.1),del(5)(q22->qter) [7]
14	M4	46,XX,t(6;11)(q27;q23),t(6;12)(p22;q23) [20]	r-*KM2TA*	46,XX,der(6)t(6;11)(q27;q23),der(6)t(6;12)(p22;q23),der(11)t(6;11)(q27;q23),der(12)t(6;12)(p22;q23) [8]
15	M1	46,XX,del(7)(?q31) [50]	del(7q)	46,XX,der(3)t(3;7)(q26;q21.1~21.2),del(7)(q21.1~21.2) [6]
16	M4/M5	44,X,-X,-7,+8,-20 [23]	r-*KM2TA*	46,XX,der(10)t(10;11)(10pter->10p12.31::11q23.3->11q23.3::10p12.31->10q11.23::14q24.2->14qter),der(11)t(11;14)(10qter->10q11.23::11p15.3->11q23.3::10p12.31->10p12.31::11q23.3->11qter),der(14)t(10;14)(q11.23;q24.2),inv(14)(q32;q11) [4]
17	M4	46,XX,der(7)t(7;?),inv(16)(p13;q22) [18]	del(7q31)	46,XX,der(7)t(7;13)(q22;q31),inv(16)(p13;q22) [7]
18	M2	49,XX,+8,+13,+17 [28]	NR	49,XX,+8,+19,+21 [10]
19	M5	53,XX,del(11q?),+mar [15]	r-*KM2TA*	53,XX,+X,+6,t(9;11)(p21.3;q23.3),+der(9)t(9;11)(p21.3;q23.3),?dup(13)(q31q34),+14,+19,+21,+22 [4]/46,XX [16]
20	M3	45,XY,-6,der(?4),del(10)(q?),t(15;17) [20]	*PML-RARα*	45,XY,-5,dic(6;9)t(6;9;10)(9pter->q12::6p25-24->q23.3::9q13-q34.1::10q25.1->qter),der(10)t(6;9;10)(10pter->q24.3::9q34.2->qter),t(15;17)(q22;q21) [6]
21	M5	48,XY,del(11q?),t(11;19),?inv(16) [21]	r-*KM2TA*	47,XY,t(11;16;19)(q23.3;p11.2;p13.3),+der(19)(16pter->16p11.2::19p13.3->19q11::19q11->19p13.3::16p11.2->16pter) [8]
22	M2	46,XX,t(8;19)(q22;?p) [23]	*RUNX1-RUNX1T1*	46,XX, t(8;19;21)(q21.3;q13.43;q22.12) [8]

Average number of abnormalities found:

- G-Banding - 3
- Molecular cytogenetics - 4

Most frequent abnormalities:

- Most involved: chromosome 21 - 9/22 patients; 6 with *RUNX1* rearrangement;
- Chromosome 8 - 7/22 patients; 5 with t(8;21) / *RUNX1-RUNX1T1*;
- Chromosome 11 - 7/22 patients; 6 with *KMT2A-r*.

Discussion and Conclusion

The characterization of cytogenetic abnormalities with FISH, M-FISH and MCB approaches were important for clinical diagnosis, revealing important prognostic information, helping in risk stratification, and in planning therapeutic approaches for the patients in this study;

In our study, the highest rate of complex karyotypes was observed in the older pediatric patient group 9/22 (41%), unlike the literature that shows the highest rate in patients < 3 years;

In this context, this study was important to discuss for the first time the prevalence of CCs and its impact on the genesis of Brazilian childhood AML. Besides, the results of this work demonstrate the efficacy of the cyto-molecular approaches in the characterization of complex karyotypes, also contributed with new findings for the literature.

Acknowledgements

This work was supported by Ministério da Saúde do Brasil, PROBRAL (DAAD No. 419/14), and FAPERJ (project No. E-26/110: 868/2013 and E-26/200.50/2016), INCT Para o Controle do Câncer, the St. Jude Children's Research Hospital (Memphis, Tenn., USA) and Center of Excellence Grant, Tenn., USA.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA