IMMUNO-MOLECULAR PROFILE OF PEDIATRIC ACUTE LEUKEMIA WITH ABERRANT MYELOID AND T PHENOTYPE AND ITS IMPACT ON THE EVENT-FREE SURVIVAL

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) with aberrant expression of myeloid markers (MY$^+$ ALL) (expression of one or two myeloid antigen) are frequent, on the other hand, leukaemia of ambigious lineage or acute biphenotypic leukaemia (BAL) is a rare and heterogeneous disease that comprise less than 5% of all acute leukemias. In this context, the European Group for the Immunological Classification of Leukaemia (EGIL) presented guidelines to classify BAL, based on a scoring system according to respective degrees of specificity of myeloid and lymphoid markers.In 2008 the World Health Organization (WHO) proposed new criteria for classification of leukaemias of ambigious lineage, introducing the term mixed phenotype acute leukaemia (MPAL),which cells express lineage-specific myeloid markers as well as lineage-specific T or B-lymphoid markers. MPAL or BAL-T/Myeloid are a rare subtype of leukemia and can be overlap with the early T-cell precursor acute lymphoblastic leukemia (ETP-ALL). ETP-ALL accounts for up to 15% of pediatric T-ALL. This kind of T-ALL has a specific immunophenotypic profile, such as: low or absent expression of CD5, lack of T-cell marker CD1a, CD8 and expression of stem cell or myeloid markers. This disease entity is associated with high risk of treatment failure and presents a transcriptional profile similar to myeloid/hematopoietic stem cell leukemia. ETP-ALL cases can be alternatively classified as MPAL/BAL-T/Myeloid. After description of ETP-ALL, several studies have been discussing the prognosis significance and how to identify these entities based on immunophenotypic criteria.

AIM

In order to understand the discrimination of both profiles and outcome relevance, we revisited the criteria to classify these subtypes and evaluated their molecular and immunophenotypic profiles and event-free survival (EFS).

PATIENTS AND METHODS

253 cases (<19 years)
(2005-2016)
cCD3 and CD7 positive
revisited for immunophenotypic analysis

Identification of MPAL/BAL T/Myeloid:
- EGIL criteria (1995) (Bene *et al*, 1995)
-WHO 2008/2016
(Borowitiz *et al*, 2008; Arber *et al*., 2016).

Identification of ETP-ALL

Screning of molecular alterations:
Sanguer Sequencing: *NOTCH1,FBXW7, IL7R, FLT3, RAS*
RT-PCR: *TLX3, STIL-TAL1,*
MLPA: *CDKN2A/B*

Myeloid fusion genes *RUNX1-RUNX1T1, CBFß-MYH11* and *KMT2A-r* were screened in cases diagnosed as MPAL/BAL T/Myeloid

Table1:EGIL Scoring system for the definition of BAL T/ Myeloid

Points	T lineage	Myeloid Lineage
2	CD3(c/m)	MPO
	TCR-αβ	anti-lysozyme
	TCR-γδ	
1	CD2	CD117
	CD5	CD13
	CD8	CD33
	CD10	CD65
0,5	TdT	CD14
	CD7	CD15
	CD1a	CD64

BAL is define when score are over 2 for myeloid and one of the lymphoid lineages

Table 2: Criteria for lineage assignment for diagnosis of MPAL T/Myeloid according WHO 2016.

Lineage assignment criteria
Myeloid lineage
MPO (Flow cytometry, or cytochemistry or
Monocytic differentiation (at least 2 of the following: non specificesterase cytochemistry, CD11c, CD14,CD64,Lysozyme
T-lineage
Strong cytoplasmic CD3 (with antibodie to CD3ε chain) or
Surface CD3

Table 3: Scoring system for immunophenotypical diagnosis of ETP-ALL based on marker expression

Score	-2	-1	+1	+2
CD5	≥ 75%			< 75%
CD8	≥ 5%			< 5%
CD13			≥ 25%	≥ 75%
CD33			≥ 25%	≥ 75%
CD34			≥ 25%	≥ 75%
HLA-DR			≥ 25%	≥ 75%
CD2		≥ 75%	< 20%	
m CD3		≥ 75%	< 20%	
CD4		≥ 75%	< 20%	
CD10		≥ 75%	< 20%	
CD56			≥ 20%	≥ 75%

ETP-ALL is defined when score is ≥ 7

Figure 1: Summary of the study design
RT-PCR: Reverse transcriptase PCR
MLPA: Mutiplex ligation probe amplification; MPAL: Mixed phenotype acute Leukemia; ETP-ALL: Early T cell acute lymphoblastic leukemia

STATISTICAL ANALYSIS

The Chi-square test or Fisher`s exact test was used to compare the distribution of categorical variables. Cases were grouped in MPAL T/Myeloid, ETP-ALL and typical T-ALL, the groups were analyzed by age range, gender, immune-molecular and clinical features to compare if any significant differences. Overall survival (OS) was measured from the date of diagnosis to the date of last follow-up or death from any cause. Event-free survival (EFS) was measured from the date of diagnosis to the date of relapse. Patients who did not experience an event were censored at the time of last follow-up and those with lost follow-up were censored at the date of last known contact. The Kaplan-Meier survival analysis method was used to calculate the 5-year of OS and EFS, and estimated survival values were compared using the log-rank test in order to verify the differences in outcome among ETP-ALL, MPAL/BAL T/Myeloid and typical T-ALL. P-values of < 0.05 were considered statistically significant. All analysis were performed using SPSS 21.0 (SPSS, Chicago, IL, USA, 2004).

RESULTS

Table 4. Demography and clinical features of MPAL/BAL T/myeloid and ETP-ALL phenotypes, Brazil, 2005-2016.

	ETP-ALL n (%)	MPAL T/Myeloid n (%)	p*	Typical T-ALL n (%)	p**
Age (years)			0.257		0.133
<10	8(34.8)	6 (60)		124 (56.4)	
10-18	15(65.2)	4 (40)		96(43.6)	
Gender			0.682		0.308
Male	15(65.2)	8(80)		174(79.1)	
Female	8 (34.8)	2(20)		46(20.9)	
Skin color			1.0		0.591
White	8(34.8)	3(30)		93(42.5)	
Non-White	15(65.2)	7(70)		126(57.5)	
WBC (x10^9/L)			0.3		0.005
< 50	11(47.8)	4(40)		57 (25.9)	
≥ 50 < 100	1(4.3)	5(50)		44 (20)	
≥100	11(47.8)	1 (10)		119 (54.1)	
Mediastinal mass			1.0		0.833
Yes	8(34.8)	4(40)		90 (41.3)	
No	15(65.2)	6(60)		128 (58.7)	
Treatment			-		-
ALL	22 (95.6)	9 (90)		220(100)	
AML	1 (4.4)	1 (10)		0	
Total	23(100)	10 (100)		220(100)	

*p-value calculated by Exact Fisher test comparing MPAL/BAL T/Myeloid and ETP-ALL. **p-value calculated by Pearson Chi-Square or Exact Fisher test comparing all subtypes.

Table 5: The immunophenotypic profile of cases classified as MPAL/BAL T/Myeloid and ETP-ALL

Case	Subtype	Myeloid Score EGIL	Score ETP Inukai	Percentage of positive cell of markers in blast gate															
				MPO	TdT	CD1a	***CD5***	***CD8***	***CD2***	***mCD3***	***CD4***	***CD10***	***CD56***	CD11b	***CD13***	***CD33***	CD117	***HLA-DR***	***CD34***
#1	ETP^{CS+IK}	1.5	10	1	89	2	74	4	0	6	2	75	53	8	73	6	72	-	98
#2	ETP^{CS+IK}	2.5	12	8	1	0	1	1	1	2	1	0	0	0	66	50	93	57	31
#3	ETP^{CS+IK}	ND	10	0	50	0	73	0	0	0	0	0	-	-	-	-	-	-	75
#4	ETP^{CS+IK}	2.5	11	1	81	0	10	0	0	1	1	0	0	10	51	45	53	41	58
#5	ETP^{CS+IK}	2.0	12	6	86	1	73	1	1	4	2	16	1	25	21	43	4	1	89
#6	ETP^{CS+IK}	0.0	8	0	52	3	64	2	3	0	0	0	0	51	0	0	11	0	1
#7	ETP^{CS+IK}	3.5	8	10	28	0	52	2	77	6	0	4	0	0	34	0	28	0	87
#8	ETP^{CS+IK}	1.5	9	0	0	5	0	0	5	15	0	0	-	3	11	27	92	0	57
#9	ETP^{CS+IK}	0.5	11	3	80	4	0	0	4	9	0	24	-	-	2	2	24	95	95
#10	ETP^{CS+IK}	1.0	9	0	76	4	54	4	4	0	0	20	-	97	0	31	11	4	49
#11	ETP^{CS+IK}	0.0	7	0	0	0	74	0	0	0	0	32	0	-	0	0	-	-	50
#12	ETP^{CS+IK}	1.0	11	0	40	2	0	0	2	1	0	7	0	51	3	37	8	16	30
#13	ETP^{CS+IK}	1.5	9	1	-	3	0	0	90	17	0	0	0	37	0	32	31	31	46
#14	ETP^{CS+IK}	0.5	10	0	-	0	70	0	0	0	0	0	0	2	0	0	83	40	29
#15	ETP^{CS+IK}	2.5	13	3	-	0	5	0	0	5	89	0	5	77	100	87	80	100	68
#16	ETP^{IK}	1.0	8	3	2	16	58	2	63	3	0	32	1	9	0	87	-	0	1
#17	ETP^{IK}	1.5	7	0	33	3	74	23	61	0	0	0	0	70	17	37	38	0	96
#18	ETP^{IK}	1.5	9	3	67	2	74	19	2	12	18	7	69	23	89	9	20	17	80
#19	ETP^{IK}	1.5	7	2	21	1	63	32	2	2	14	4	3	32	48*	48*	26	3	72
#20	ETP^{IK}	ND	9	0	80	5	72	5	6	1	12	3	-	-	-	0	-	-	60
#21	ETP^{IK}	1.0	7	0	84	9	78	4	3	1	28	9	0	2	23	0	1	8	18
#22	ETP^{IK}	1.0	7	0	14	13	90	5	10	0	0	9	-	98	0	94	7	27	80
#23	ETP^{IK}	1.0	9	0	-	0	4	3	46	33	0	0	0	9	0	91	2	0	78
#24	MPAL/BAL	4.0	4	91	3	11	78	7	31	5	3	5	-	47	51	50	14	26	70
#25	BAL	3.0	4	1	46	1	97	42	96	3	12	-	95	74	95	28	95	92	-
#26	MPAL/BAL	2.5	7	40	20	11	16	15	17	0	-	0	7	11	4	0	32	17	-
#27	MPAL/BAL	3.0	1	51	80	3	71	83	78	3	85	0	0	4	10	73	1	5	7
#28	MPAL/BAL	3.0	2	76	83	69	5	89	68	10	80	70	2	3	2	89	1	0	1
#29	MPAL/BAL	3.0	6	66	38	0	42	49	39	63	26	6	13	75	87*	87*	-	72	1
#30	MPAL/BAL	3.5	12	54	60	13	2	0	96	2	2	0	-	41	73	6	93	92	90
#31	MPAL/BAL	2.5	9	0	90	0	2	0	100	20	0	-	0	0	85	46	100	100	100
#32	MPAL/BAL	4.0	3	67	-	2	93	0	94	44	0	-	0	93	77	61	0	4	66
#33	MPAL/BAL	4.5	12	45	-	0	0	0	43	0	2	0	0	35	80	38	46	39	48

ETP^{CS+IK}, ETP-ALL identified by Counsthan Smith *et al*, 2009 and Inukai *et al*, 2011 criteria; ETP^{IK}, ETP-ALL identified only by Inukai *et al*, 2011 criteria; In ***italic and bold***, markers that were considered to calculate score proposed by Inukai *et al*, 2011. MPAL/BAL $^{T/M}$, mixed phenotype acute leukemia or acute biphenotypic leukaemia T/myeloid;
m, membrane, *CD13 e CD33 marked in the same tube; All cases of ETP-ALL were positive to cytoplasmic CD3 in more than 35% of cells and CD7

Table 6. The differences of immunophenotype profile of cases classified as MPAL/BAL T/Myeloid,. ETP-ALL.

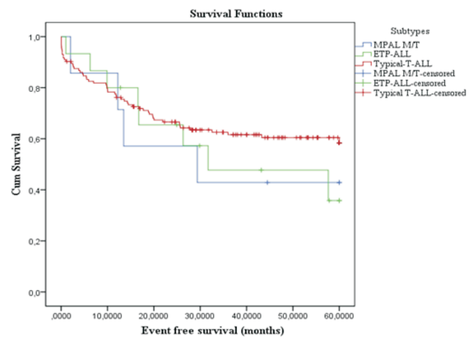
Marker	Total n (%)	MPAL/BAL T/Myeloid	ETP-ALL	p*
CD34	82/233(35.2)	5/8(62.5)	20/23(87.0)	0.161
HLA-DR	31/208 (14.9)	8/9(88.9)	7/19 (36.8)	***0.016***
CD117	27/165 (16.4)	5/9(55.6)	12/18 (66.7)	0.683
TdT	157/208 (75.5)	7/8(87.5)	15/20(75)	0.640
CD10	82/231(35.5)	1/7(14.3)	5/23(21.7)	1.0
CD11b	31/157(19.7)	6/10 (60)	10/19(52.6)	1.0
CD13/CD33	63/187(33.7)	9/10(90)	17/21(81)	1.0
CD56	17/141(12.1)	1/9(11.1)	2/15(13.3)	1.0
T cell markers				
CD1a	99/253 (39.1)	1/10 (10)	0/23	0.303
CD2	186/240 (77.5)	9/10 (50)	7/23 (30.4)	***0.002***
CD5	223/244(91.4)	5/10(50)	15/23(65.2)	0.461
mCD3	134/252(53.2)	3/10(30)	1/23(4.3)	0.073
CD4	133/243(54.7)	3/9(33.3)	1/23(4.3)	0.057
CD8	163/250(65.2)	4/10(40)	2/23(8.7)	0.053

n-Number of cases positive/number of case tested. CD3 intracytoplasmatic and CD7 were positive in all cases.*p-value calculated by Exact Fisher test comparing MPAL T/Myeloid and ETP-ALL.

Table 7. The frequency of molecular alteration in MPAL/ BAL T/Myeloid, ETP-ALL and Typical T-ALL

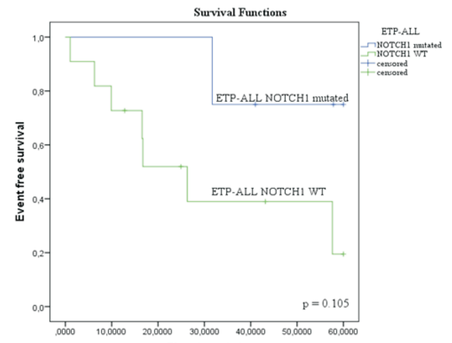
Molecular alteration	n (%)	MPAL /BAL T/Myeloid	ETP-ALL	*p	Typical T-ALL	**p
NOTCH1^{mut}	91/198(46)	2/9(22.2)	10/23(43.5)	0.422	79/166(47.6)	0.320
FBXW7^{mut}	31/197(15.7)	0/9	3/23(13)	0.541	28/165(17)	0.369
N/KRAS^{mut}	19/202(9.4)	2/9(22.2)	2/22(9.1)	0.560	15/171(8.8)	0.403
FLT3^{mut}	9/196(4.6)	1/9(11.1)	5/22(22.7)	0.642	3/165(1.8)	**<0.001**
IL7R ^{mut}	13/195(6.7)	0/9	0/22	-	13/164(7.9)	0.268
STIL-TAL1r	42/201(20.9)	0/5	3/20(15)	1.0	38/176(21.6)	0.408
TLX3r	22/215(10.2)	1/6(16.7)	1/19(5.3)	0.430	20/190(10.5)	0.671
CDKN2AB^{del}	99/143(69.2)	0/4	4/19(21.1)	1.0	95/120(79.2)	**<0.001**

n- Number of cases with molecular alteration /number of case tested. Del-deleted, mut-mutated, r- rearranged;*p-value calculated by Exact Fisher test comparing MPAL T/Myeloid and ETP-ALL;**p-value calculated by Pearson Chi-Square or Exact Fisher test comparing all subtypes.



Subtypes	NE/TN	EFS% (CI95%)
MPAL/BAL T/Myeloid	4/7	33.9 (16.2-51.5)
ETP-ALL	6/15	37.1 (25.1- 49.1)
Typical T-ALL	51/144	40.9 (36.7-45)

Figure 2: The survival curves in 60 mouths of MPAL Myeloid/T, ETP-ALL and Typical T-ALL. NE, N of events; TN, Total N; OS, overall survival; EFS, event free-survival; CI, confidential interval .



Subtypes	NE/TN	EFS% (CI95%)
ETP-ALL ***NOTCH1***^{Mut}	1/4	52.9% (40.9-64.9)
ETP-ALL ***NOTCH1***^{WT}	7/11	31.3% (16.9-45.8)

Figure 3: The survival curve in 60 mouths of ETP-ALL ***NOTCH1***^{Mut} and ETP-ALL ***NOTCH1***^{WT}.NE, N of events; TN, Total N; OS, overall survival; EFS, event free-survival; CI, confidential interval

CONCLUSION

Our results suggest that the molecular profile of ETP-ALL and MPAL/BAL-T/Myeloid should be taken into consideration to assist the risk stratification and treatment decisions.

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