QUALITATIVE AND QUANTITATIVE FLOW CYTOMETRY ANALYSIS TO EVALUATE NG2 ANTIGEN AS A MARKER FOR *MLL* DIAGNOSIS

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INTRODUCTION

Chromosomal rearrangements of the *MLL* gene are a hallmark for studies in early childhood acute leukemia (AL). Most leukemic cells carrying *MLL*$^{+}$ in both acute lymphoblastic (ALL) and myeloid (AML) leukemia cases express NG2 homologue, a chondroitin sulfate molecule, which reacts with the monoclonal antibody (MoAb) 7.1 that can be detected by flow cytometry (FC). FC is a widely accepted diagnosis method that provides accurate identification of leukemia lineage and subclasses.

AIM

To test the potential of FC in the diagnosis of MLL abnormalities (MLL+rear) in AL, based on the expression of NG2 antigen, through qualitative (QL) and quantitative (QT) measurements.

MATERIAL AND METHODS

- ✓ Bone marrow aspirates from 120 children with AL were evaluated by FC previously to *MLL*$^{+rear}$ analysis [2003-2009] (Figure 1);
- ✓ Immunophenotyping was performed with three-color MoAbs combinations, being NG2 detected by the CD34/7.1/CD45 combination;
- ✓ QL (negative, low, bright signals) and QT (molecules of equivalent soluble fluoresceinMESF) database was explored using univariated method;
- ✓ *MLL*$^{+rear}$ was screened by conventional cytogenetics, RT-PCR and FISH as 'gold standard' (GS) reference.

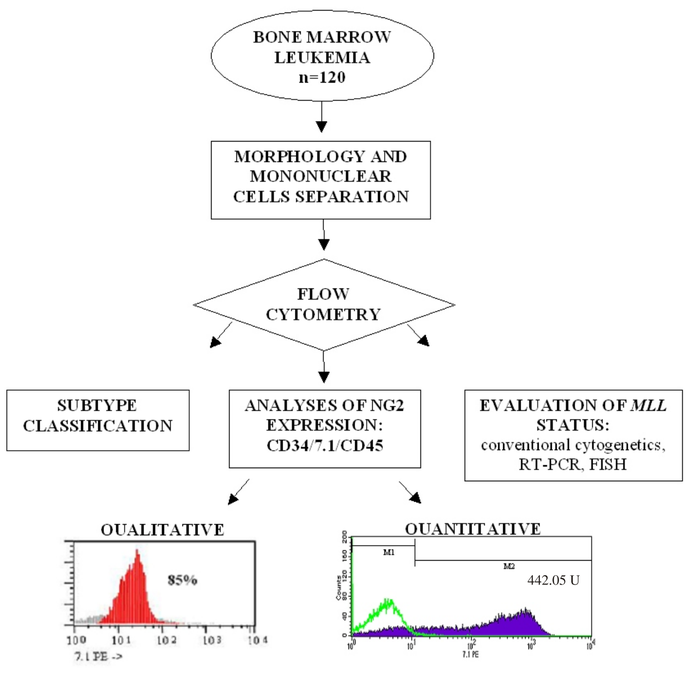

Figure 1. Schematic view of materials and methods

RESULTS

- ✓ There were 63 boys and 38 girls, median age 11.5 (0-24 months);
- ✓ 42 (36.2%) were classified as pro-B acute lymphoblastic leukemia (ALL), 27 (23.2%) common ALL, 2 (1.7%) pre-B ALL, 4 (3.4%) pro-T ALL and 34 (29.3) as acute myeloid leukemia (AML) (Table 1);
- ✓ To test how well NG2 analyses match *MLL* status, we calculated sensitivity and specificity of all *MLL*$^{+rear}$ detection methods combined (n=115) and of only GS method (n=37) (Table 2);
- ✓ The results show significant differences between *MLL*$^{+rear}$ detection methods, indicating that GS technique increases both the identification of true *MLL*$^{+rear}$ patients (sensitivity: 0.71 vs 0.78) and the identification of germline *MLL* patients (specificity: 0.73 vs 0.92) by FC;
- ✓ We next performed the comparison between QL/QT-FC results analyzing cases with *MLL* status defined by the GS method. Both sensitivity (0.82 vs 0.78) and specificity (0.93 vs 0.92) results were slightly better using QT-FC, but without significance (Table 2 and Figure 2);
- ✓ Positive predictive value (PPV) and negative predictive value (NPV) have been retrieved for each FC approach: no significant differences were shown between the two methods considering both PPV (0.90 vs 0.94) and NPV (0.93 vs 0.92) QT and QL outcomes, respectively.

Table 1. Distribution of cases according to phenotype

Subtypes	n	%	*MLL(+)*	*MLL(-)*
pro-B ALL	42	36.2	28	12
common-ALL	27	23.2	10	16
pre-B ALL	2	1.7	0	2
pro-T ALL	4	3.4	2	2
AML	34	29.3	15	23

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; 12 cases of ALL were not subclassified

Table 2. Comparison between flow cytometry and *MLL* diagnosis using gold-standard method

	***MLL* all methods combined**		***MLL* gold-standard**	
	QL FC	**QN FC**	**QL FC**	**QN FC**
True positive	41/56 (73%)	22/31 (70,9%)	19/25 (76%)	17/21 (81%)
True negative	43/59 (73%)	28/38 (73.6%)	11/12 (91.6%)	10/12 (83.3%)
False positive	16	10	1	2
False negative	15	9	6	4
Sensitivity	0.73	0.71	0.76	0.81
Specificity	0.73	0.74	0.92	0.83
Positive predictive value	0.73	0.69	0.91	0.95
Negative predictive value	0.74	0.76	0.90	0.92

Abbreviations: FC, flow cytometry; QL, qualitative; QN, quantitative

QL FC vs GS	QN FC vs GS	
1	2	*FP*
19	17	*TP*
6	4	*FN*
11	10	*TN*

Figure 2. Comparison between flow cytometry (FC: QL = qualitative, QN quantitative) and *MLL* diagnosis using gold-standard (GS) method; FP = false positive; TP = true positive; FN = false negative e TN = true negative

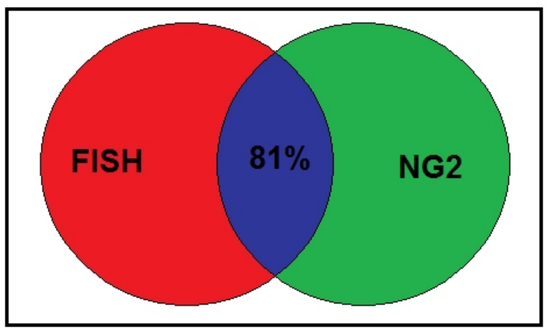

Figure 3. Concordance between FISH and quantitative flow cytometry detection of *MLL* rearranged cases

CONCLUSION

Immunophenotyping is a reliable approach to identify MLL status in AL, although cytogenetic and molecular methods should always be performed.