

# The utility of flow cytometry for the detection of tumor cells in cerebrospinal fluid of patients with acute leukemia

Ximena Torres<sup>1,5</sup>, Iliana De Los Reyes<sup>2</sup>, Martha Vizcaíno<sup>2</sup>, Paula Carolina Guzmán<sup>2</sup>, Alba Campos<sup>1</sup>, Niyireth Peñalosa<sup>1</sup>, Ana María Uribe<sup>3</sup>, Carlos Saavedra<sup>4</sup>, Martha Romero<sup>4</sup>, Gina Cuéllar<sup>4</sup>, Liliana Martín<sup>4</sup>, Paula Rodríguez<sup>4</sup>, Claudia Cardozo<sup>1</sup>, Sandra Quijano\*<sup>1,4,5</sup>

**Table 1.** Panel of antibodies used for the diagnosis of CSF infiltration for ALL.

	FITC	PE	PerCPCy5	PECy7	APC	APCCy7
<b>T-cell ALL</b>						
Tube 1	nTdT or CD7	CD34	cyCD3	sCD3	CD14	CD45
Tube 2	CD8	CD4	CD3	-	CD56	CD45
<b>B-cell ALL</b>						
Tube 1	CD34	CD10	CD19	sCD3	CD14	CD45
Tube 2	CD8	CD4	CD3	-	CD56	CD45
<b>AML</b>						
Tube 1	CD34	CD117	CD19	sCD3	CD14	CD45
Tube 2	CD8	CD4	CD3	-	CD56	CD45
<b>Volumes</b>	5 µL	4 µL	4 µL	3 µL	3 µL	2 µL

FITC (Fluorescein isothiocyanate); PE (Phycoerythrin); PerCPCy5 (Peridinin-chlorophyll proteins-Cyanine 5); PECY7 (Phycoerythrin-cyanine 7); APC (Allophycocyanin); APC-Cy7 (Allophycocyanin-Cyanine 7). All antibodies purchased from BD (Becton Dickinson & Company).

**Table 2.** Frequency of patients with CNS tumor infiltration detected by FCM analysis of CSF.

Phenotype	n	Moment of the evaluation	
		Diagnosis	Follow-up
B-cell ALL	37	3 (8 %)	7 (19 %)
T-cell ALL	8	1 (12 %)	1 (12 %)
AML	8	1 (12 %)	2 (25 %)
Mixed-phenotype	2	1 (50 %)	0
<b>TOTAL</b>	<b>55</b>	<b>6 (11 %)</b>	<b>10 (18 %)</b>

**Table 3.** Cell subsets in CSF of patients with B-cell ALL according to CNS tumor infiltration by FCM.

VARIABLE	Infiltration ( <i>n</i> = 17)		No infiltration ( <i>n</i> = 72)		<i>p</i> *
	Mean	Range	Mean	Range	
Total cell number $\mu\text{L}^{-1}$	893	0.1 to 13 461	11.4	0.01 to 220	< 0.001
T-cells $\mu\text{L}^{-1}$	25.4	0.03 to 229	5.3	0.01 to 74	0.004

\* Mann-Whitney test. Detection limit of FCM was 0.07 cells  $\mu\text{L}^{-1}$ .

**Table 4.** Prognosis factors in patients with T-cell ALL according to CNS infiltration detected by FCM analysis of CSF.

FACTOR	FCM–	FCM+	<i>p</i>
<b>At diagnosis</b>			
White blood cells in peripheral blood ( $1 \times 10^3 \mu\text{L}^{-1}$ )	105 (5-259)	135.5 (26-245)	NS
Blasts in bone marrow (%)	75 (39-96)	80 (78-83)	NS
Blasts in peripheral blood (%)	58 (2-96)	53 (12-94)	NS
Platelets ( $1 \times 10^3 \mu\text{L}^{-1}$ )	143 (36.7-271)	24 (20-28)	0.053
Neutrophils ( $1 \times 10^3 \mu\text{L}^{-1}$ )	6 (1.6-13)	24 (5.6-42.3)	NS
Haemoglobin (g dL <sup>-1</sup> )	11 (6.7-16.2)	10.1 (8.2-12)	NS
LDH (UI/L)	8533 (847-21 500)	6142 (3166-9119)	NS
<b>Cytogenetic findings<sup>b</sup></b>			
Normal	3/4	2/2	NS
Hyperdiploid	1/4	0/2	
<b>Risk-group assignment<sup>a</sup></b>			
Low	0/5	0/2	NS
Intermediate	0/5	1/2	
High	5/5	1/2	
<b>Extramedullary infiltration<sup>a</sup></b>			
Yes	0/7	2/2	0.014
No	7/7	0/2	
<b>Neurological symptoms<sup>a</sup></b>			
Yes	0/5	0/2	NS
No	5/5	2/2	
<b>Treatment response</b>			
Blast count on day 8 (blast $\mu\text{L}^{-1}$ ) <sup>a</sup>	1564 (243-3800)	41730 (20-83 440)	NS
<b>Complete remission<sup>b</sup></b>			
Yes	4/4	2/2	NS
No	0/4	0/2	
<b>Relapse<sup>b</sup></b>			
Yes	5/5	1/2	NS
No	0/5	1/2	
<b>Death<sup>c</sup></b>			
Yes	0/5	1/2	NS
No	5/5	1/2	

Mann-Whitney test. NS: non-significant. Death: Yes (dead); No (alive), death caused by the progression of disease. Values between parentheses correspond to ranges, and values outside of parentheses correspond to means. Bold data: statistically significant.

<sup>a</sup> Prednisolone response, sample size for FCM+ = 2 and FCM– = 4.

<sup>b</sup> Sample size FCM+ = 2 and FCM– = 5.

<sup>c</sup> Sample size for FCM+ = 2 and FCM– = 4.

**Table 5.** Prognosis factors in patients with AML according to CNS infiltration detected by FCM analysis of CSF.

FACTOR	FCM–	FCM+	<i>p</i>
<b>At diagnosis</b>			
White blood cells in peripheral blood ( $1 \times 10^3 \mu\text{L}^{-1}$ )	112 (6.7–286)	35 (5.2–69)	NS
Blasts in bone marrow (%)	21 (15–39)	48.3 (5.9–82)	NS
Blasts in peripheral blood (%)	32 (7–67)	40.6 (30–52)	NS
Haemoglobin ( $\text{g dL}^{-1}$ )	9 (6.1–13.7)	12 (9.1–17.1)	NS
Platelets ( $1 \times 10^3 \mu\text{L}^{-1}$ )	60 (21–131)	92 (27–149)	NS
Neutrophils ( $1 \times 10^3 \mu\text{L}^{-1}$ )	47.4 (2.2–179)	10.5 (2.7–17.1)	NS
LDH (UI/L)	1997 (893–2851)	2 624 (1.050–4892)	NS
<b>Cytogenetic findings</b>			
<i>t</i> (8;21)	1/4	1/3	NS
GATA	1/4	1/3	
Normal	1/4	0/3	
Hyperdiploid	1/4	1/3	
<b>Risk-group assignment</b>			
Favourable	1/5	1/3	NS
Intermediate	0/5	1/3	
Unfavourable	4/5	1/3	
<b>Extramedullary infiltration</b>			
Yes	1/5	3/3	0.04
No	4/5	0/3	
<b>Neurological symptoms</b>			
Yes	0/5	1/3	NS
No	5/5	2/3	
<b>Treatment response</b>			
Complete remission			
Yes	3/4	1/2	NS
No	1/4	1/2	
Relapse			
Yes	2/4	1/3	NS
No	2/4	2/3	
Death			
Yes	2/5	1/3	NS
No	3/5	2/3	

Mann-Whitney test. NS: non-significant. Death: Yes (dead); No (alive), death caused by the progression of disease. Values between parentheses correspond to ranges, and values outside of parentheses correspond to means. Bold data: statistically significant.

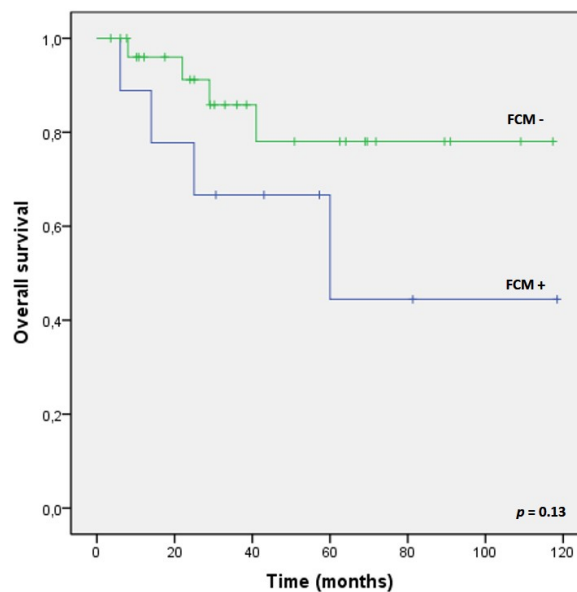
<sup>a</sup> Sample size applicable for the blood count and LDH variables, FCM+ = 3 and FCM– = 5.

**Table 6.** Multivariate Cox model for relapse-free survival ( $n = 12$ ) and overall survival ( $n = 10$ ) of AL patients with known data on all covariates in the model.

Variable	Relapse-free survival	Overall survival
	<i>p</i>	<i>p</i>
White blood at diagnosis ( $> 50\,000\ \mu\text{L}^{-1}$ )	0.12	0.07
Complete remission	$< 0.001$	0.001
Age	0.12	0.11
Immunophenotype	0.78	0.52
CNS Infiltration by FCM	$< 0.001$	$< 0.001$
<b>Risk</b>		
Low	0.07	0.18
Intermediate	0.58	0.20
High	0.11	0.14
<b>Cytogenetics</b>		
$t(9; 22)$	0.01	0.01
$t(4; 11)$	0.10	0.53
$t(12; 21)$	0.28	0.36
$t(1; 19)$	0.96	0.88
Normal cariotype	0.22	0.92

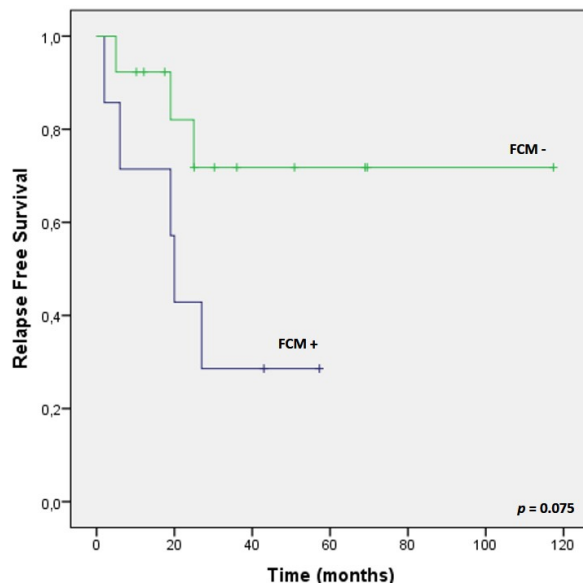
Abbreviations:

CNS: central nervous system. FCM: flow cytometry.



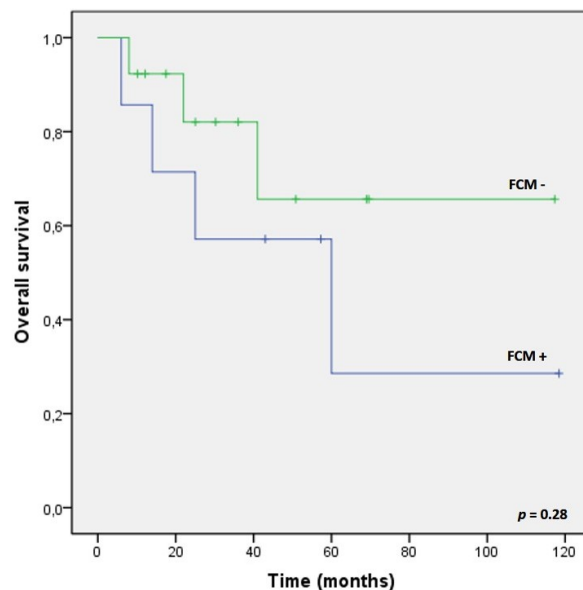
	Patients (n)	Death (n)	OS (mean in months)
FCM +	9	4	70
FCM -	28	4	97

**Figure 1.** Overall survival of patients with B-cell ALL, with (blue line) or without (green line) CNS tumor infiltration detected by FCM. Log-rank test (Mantel-Cox)  $p = 0.13$ .



	Patients (n)	Relapse (n)	RFS (mean in months)
FCM +	7	5	27
FCM -	13	3	89

**Figure 2.** Relapse-free survival of high-risk B-cell ALL patients, with (blue line) or without (green line) CNS tumor infiltration detected by FCM. Log-rank test (Mantel-Cox)  $p = 0.075$ .



	Patients (n)	Death (n)	OS (mean in months)
FCM +	7	4	57
FCM -	13	3	86

**Figure 3.** Overall survival of high-risk B-cell ALL patients, with (blue line) or without (green line) CNS tumor infiltration detected by FCM. Log-rank test (Mantel-Cox)  $p = 0.28$ .