Tatiana Verzhbitskaya and Alexander Popov

Detection and Monitoring of CNS Involvement in Children with Acute Leukemia by Flow Cytometry

Pediatric Oncology/Hematology Center, Regional Children Hospital, Ekaterinburg, Russia

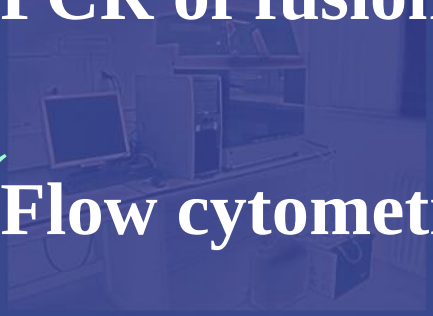
Research Institute of Medical Cell Technologies, Ekaterinburg, Russia

Ural State Medical Academy, Ekaterinburg, Russia

«Haematopoiesis Immunology», June 7-9 2010, Moscow

Methods Used for Leukemic Cells Detection in CSF

- ✓ Conventional microscopy of cytospin slides
- ✓ Immunocytology
- ✓ FISH
- ✓ PCR of fusion genes and IgH/TCR rearrangements
- ✓ Flow cytometry



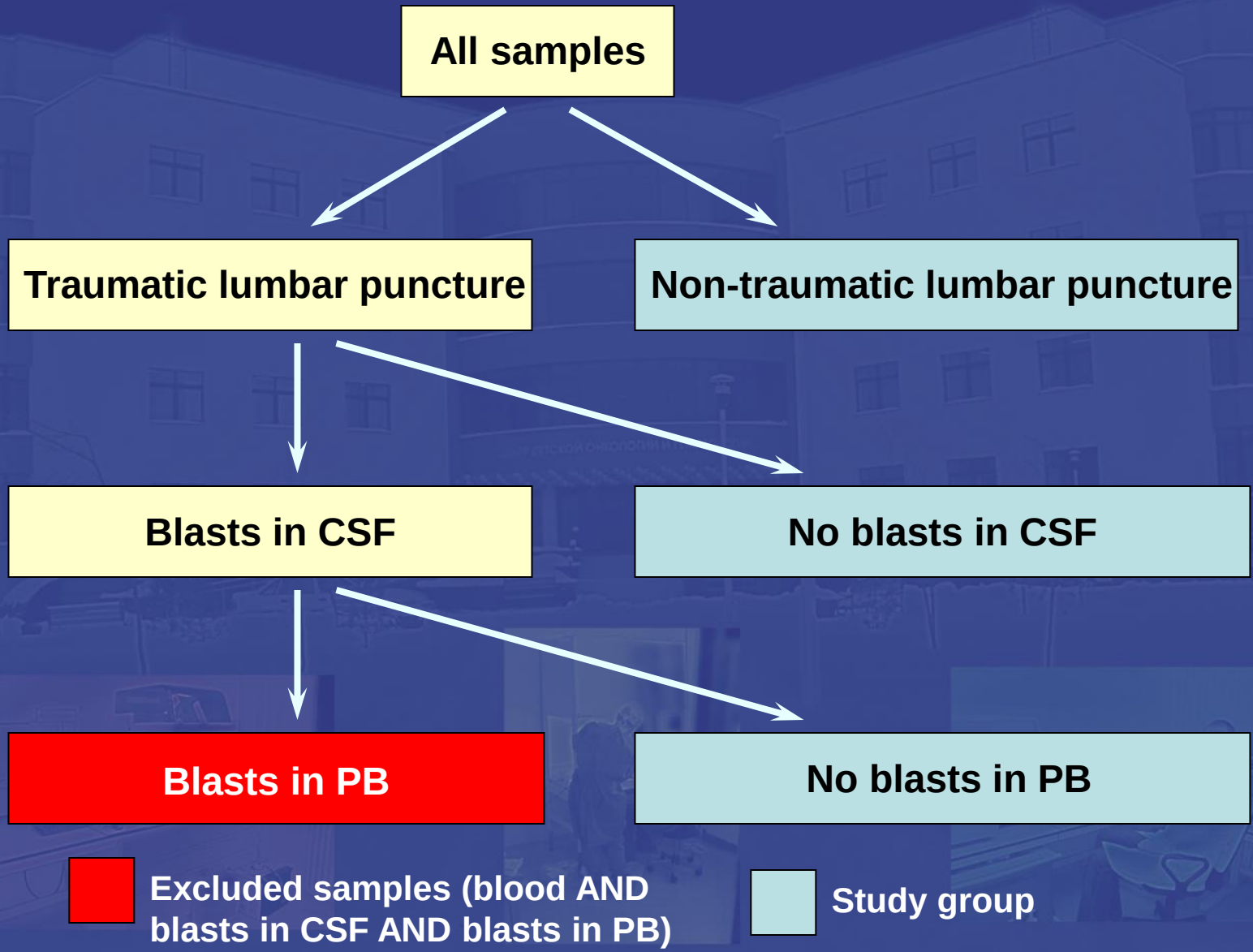
D. Subira, S. Castañón, A. Román, E. Aceituno,
C. Jiménez-Garófano, A. Jiménez, R. García and M. Bernácer

Flow Cytometry and the Study of Central Nervous Disease in Patients with Acute Leukaemia

British Journal of Haematology, 2001, Vol. 112, p. 381-384



Samples Selection in Our Study



Patients and Samples

- ✓106 CSF samples from 53 children with AL (35 male and 18 female)
- ✓40 patients with BII-ALL, 1 – BI-ALL, 8 – T-ALL, 4 - AML

Samples

	Initial diagnostics	During therapy	Relapse. Time of diagnostics	Relapse. During therapy	Total
BII-ALL	31	6	6	17	60
BI-ALL	1	0	0	0	1
T-ALL	4	11	4	20	39
AML	1	1	3	1	6
Total	37	18	13	36	106

Monoclonal Antibodies

- ✓ FITC –CD45, CD58, CD99, CD7, CD65
- ✓ PE –CD10, CD34, CD7, CD133, CD45, CD22, CD1a, NG2
- ✓ PerCP – CD8, CD20
- ✓ PerCP-Cy5.5 – CD19, CD33
- ✓ PE-Cy7 – CD34, CD3, CD56, CD13
- ✓ APC –CD117, CD133, CD3, CD19, CD56
- ✓ APC-Cy7 – CD45

BD FACS Canto II 2 lasers, 6 colors

Cytospin Slides Preparation

Leukemic sells detection in CSF in BCP-ALL

Patient G.

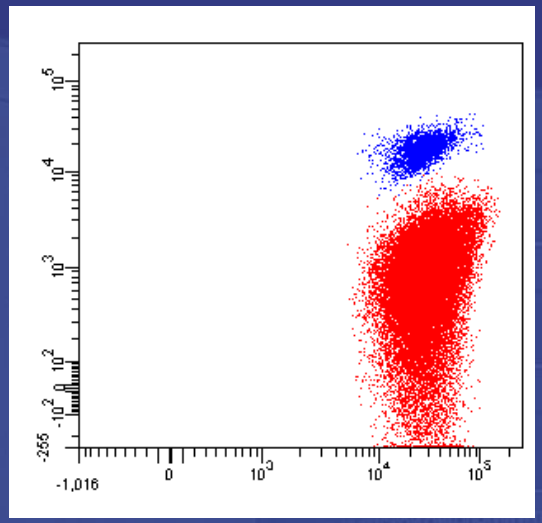
BM

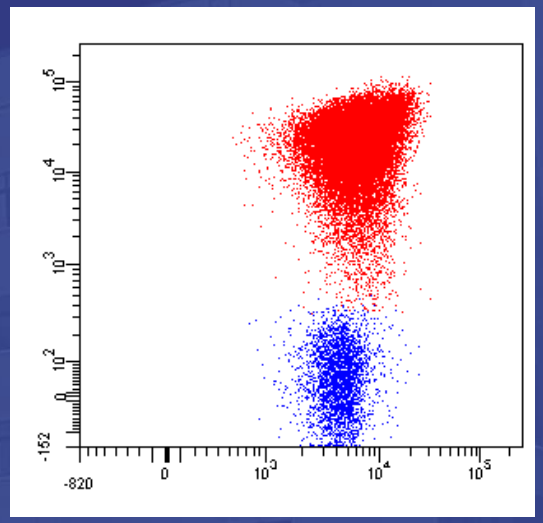
CSF

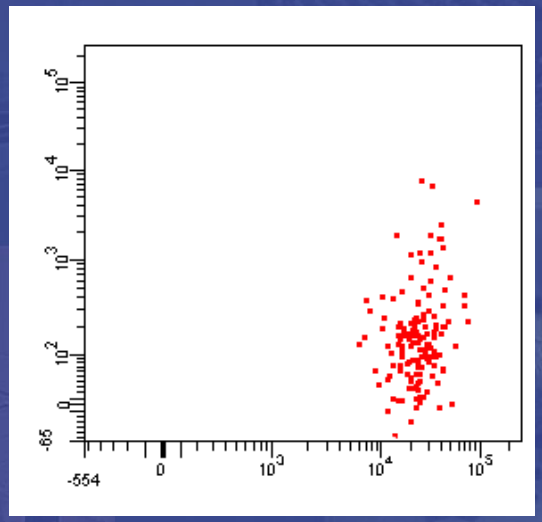
CD45

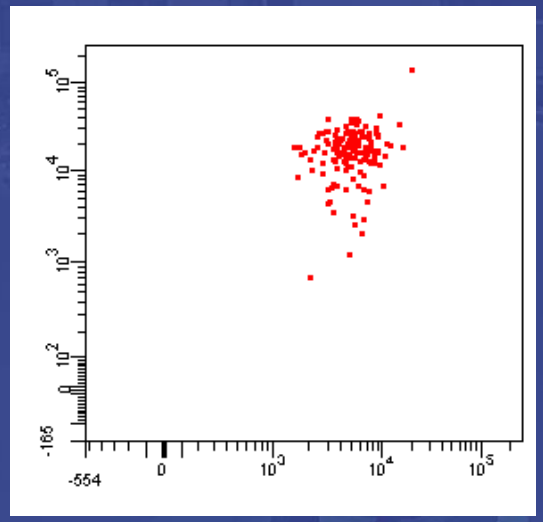
CD34

CD19

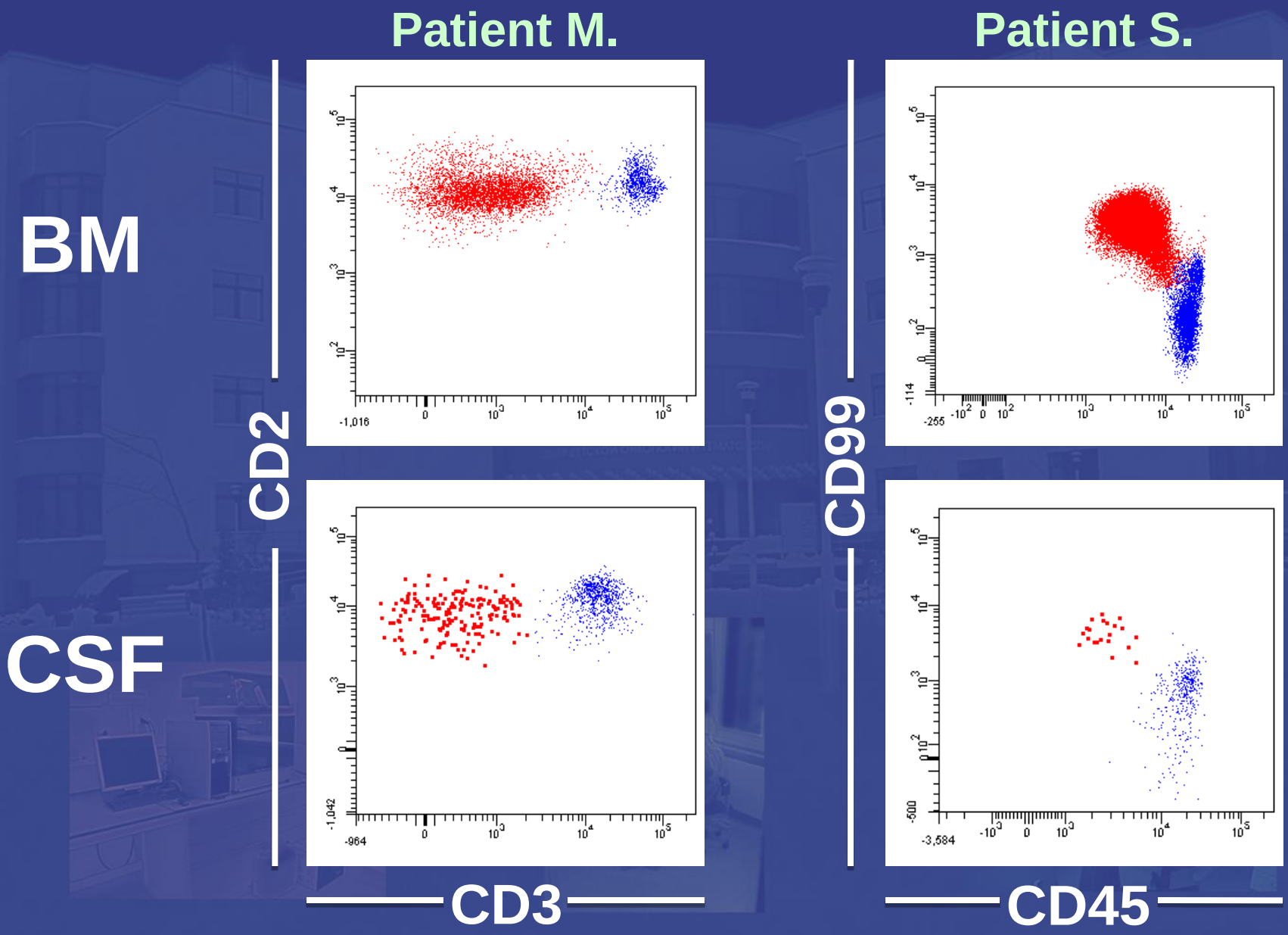








Leukemic sells detection in CSF in T-ALL



Leukemic sells detection in CSF in AML

Patient D.

BM

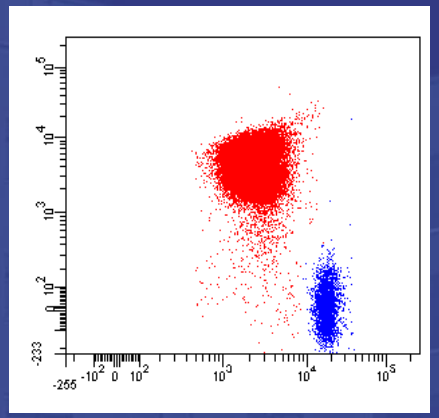
CSF

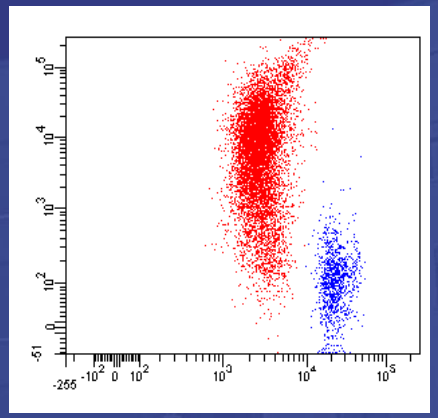
CD33

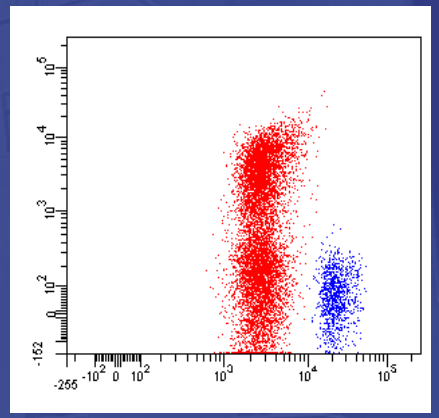
CD65

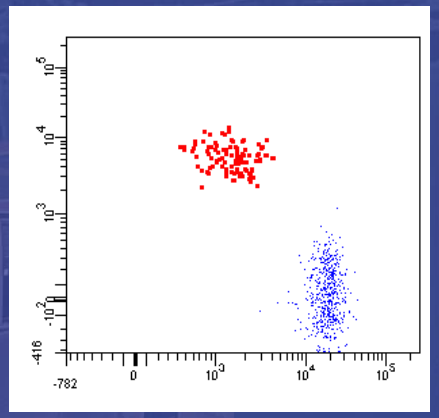
NG2

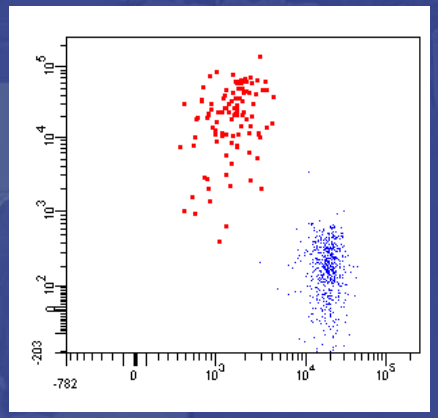
CD45

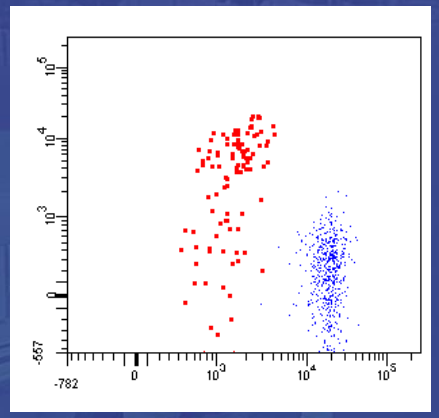












Results

All samples

		Microscopy		Total
		Positive	Negative	
Flow cytometry	Positive	17	26	43
	Negative	0	63	63
	Total	17	89	106

$p < 0{,}001$

Results

Initial samples

		Microscopy		Total
		Positive	Negative	
Flow cytometry	Positive	7	5	12
	Negative	0	25	25
	Total	7	30	37

Results

Relapse diagnostics samples

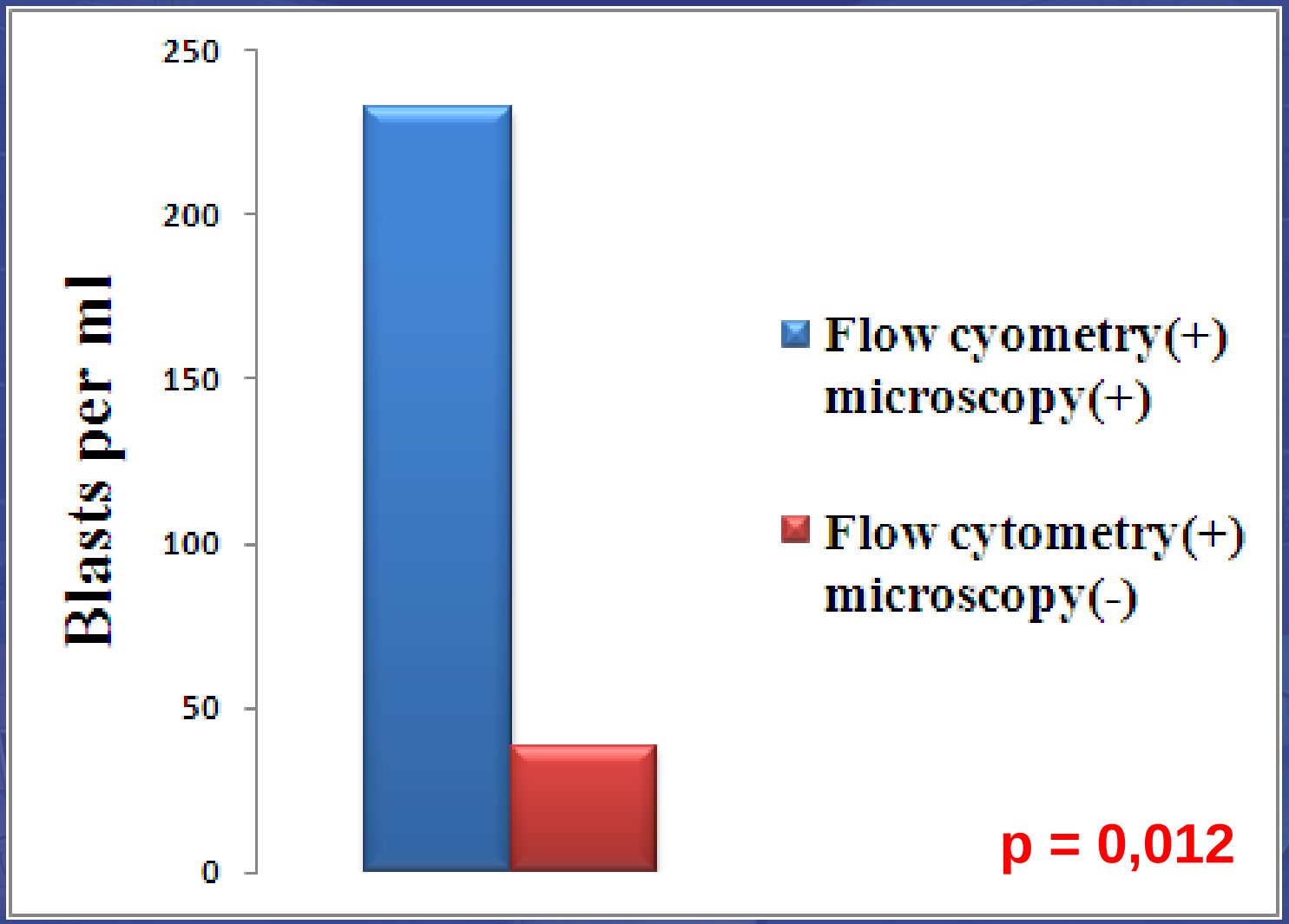
		Microscopy		Total
		Positive	Negative	
Flow cytometry	Positive	4	3	7
	Negative	0	6	6
	Total	4	9	13

Results

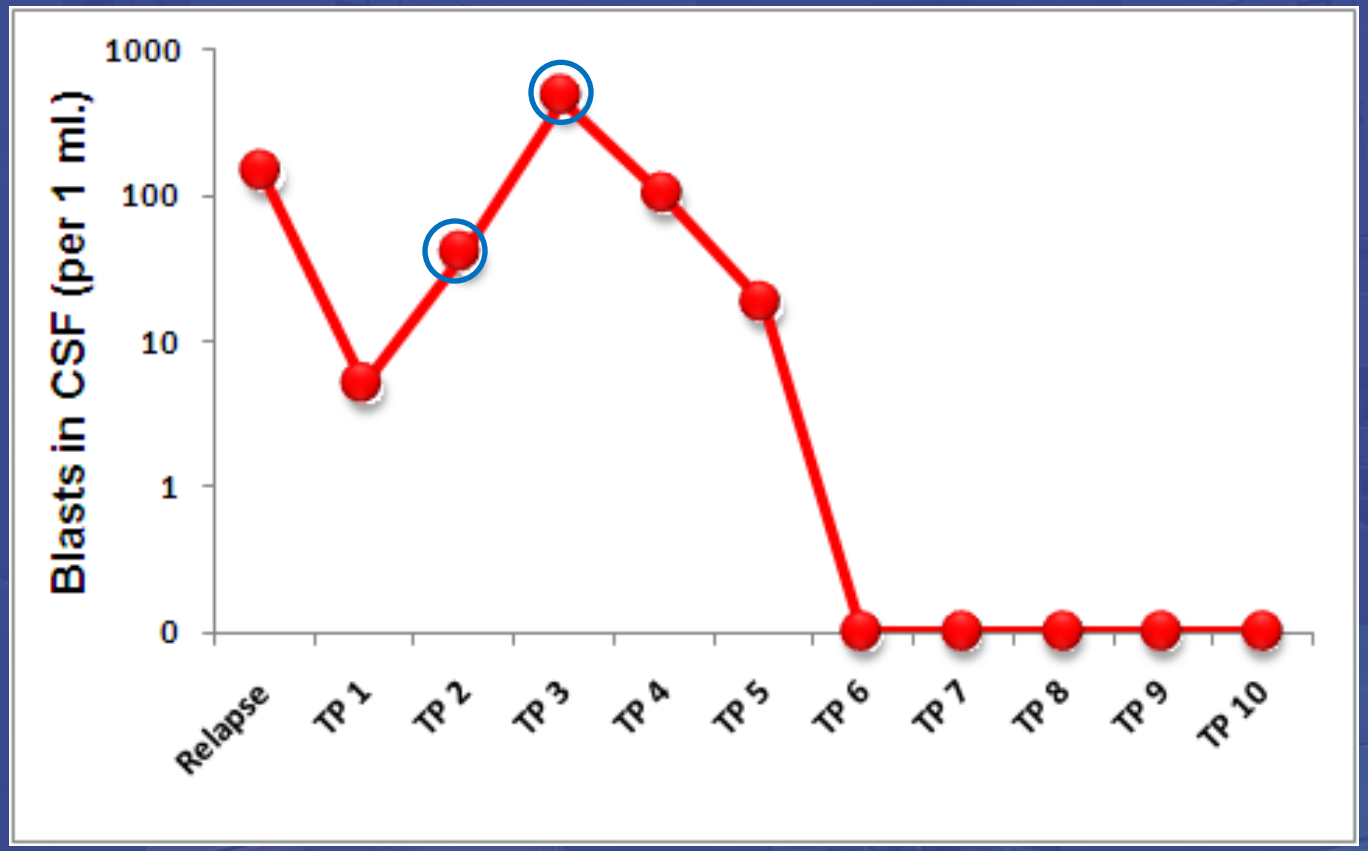
Follow-up samples

		Microscopy		Total
		Positive	Negative	
Flow cytometry	Positive	6	18	24
	Negative	0	32	32
	Total	6	50	56

Median Blast Count in Positive CSF Samples



CSF Blast Kinetics in Relapsed BCP-ALL Patient



- blasts were also detected by microscopy

Risk groups stratification in patients with discordant results in initial and relapse diagnostics samples

	Stratified without flow cytometry data	If intended to stratify with consideration of flow cytometry data
Primary ALL (stratified according to ALL-MB-2008)		
Pt. 1	SR	ImR
Pt. 2	ImR	ImR
Pt. 3	ImR	ImR
Pt. 4	ImR	ImR
Pt. 5	ImR	ImR
Relapsed ALL Primary ALL (stratified according to ALL-BFM-REZ)		
Pt. 6	S2	S2
Pt. 7	-*	-
Relapsed AML (stratified according to AML-BFM-REZ)		
Pt. 8	CNS(+)**	CNS(+)

* Non-program treatment in patient with 3rd relapse
** Tumor mass in brain detected by MRI

Conclusions

- ✓ Flow cytometry allows to detect leukemic cells in CSF more frequently than conventional microscopy of cytospin slides

- ✓ In order to evaluate prognostic significance of flow cytometric CNS involvement detection we need further investigation in large clinical trials with longer follow-up

Thank You for Your Attention!