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

Ximena Torres¹,⁵, Iliana De Los Reyes², Martha Vizcaíno², Paula Carolina Guzmán², Alba Campos¹, Niyireth Peñalosa¹, Ana María Uribe³, Carlos Saavedra⁴, Martha Romero⁴, Gina Cuéllar⁴, Liliana Martín⁴, Paula Rodríguez⁴, Claudia Cardozo¹, Sandra Quijano*¹,⁴,⁵

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

| Phenotype  | Moment of the evaluation | n  | Volumes |
|------------|--------------------------|----|---------|
|            | Diagnosis                | 37 | 5 µL    |
|            | Follow-up                | 7  | 4 µL    |
| B-cell ALL |                          | 8  | 4 µL    |
| T-cell ALL |                          | 8  | 4 µL    |
| AML        |                          | 8  | 4 µL    |
| Mixed-phenotype |                  | 2  | 3 µL    |
| TOTAL      |                          | 55 | 2 µL    |

FITC (Fluorescein isothiocyanate); PE (Phycerythrin); PerCPCy5 (Peridinin-chlorophyll proteins-Cyanine 5); PECY7 (Phycerythrin-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  | 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         |
| TOTAL          | 55 | 6 (11 %)  | 10 (18 %) |
Table 3. Cell subsets in CSF of patients with B-cell ALL according to CNS tumor infiltration by FCM.

| VARIABLE                  | Infiltration (n = 17) | No infiltration (n = 72) | p*     |
|---------------------------|-----------------------|--------------------------|--------|
|                           | Mean  | Range       | Mean  | Range       |        |
| Total cell number µL⁻¹    | 893   | 0.1 to 13 461 | 11.4  | 0.01 to 220 | < 0.001|
| T-cells µL⁻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 µL⁻¹.

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

| FACTOR                           | FCM−  | FCM+  | p     |
|----------------------------------|-------|-------|-------|
| At diagnosis                     |       |       |       |
| White blood cells in peripheral blood (1 x 10³ µL⁻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 x 10³ µL⁻¹)         | 143 (36.7-271) | 24 (20-28) | 0.053 |
| Neutrophils (1 x 10³ µL⁻¹)       | 6 (1.6-13)  | 24 (5.6-42.3) | NS |
| Haemoglobin (g dL⁻¹)             | 11 (6.7-16.2) | 10.1 (8.2-12) | NS |
| LDH (UI/L)                       | 8533 (847-21 500) | 6142 (3166-9119) | NS |
| Cytogenetic findings³            |       |       |       |
| Normal                           | 3/4   | 2/2   | NS    |
| Hyperdiploid                     | 1/4   | 0/2   |       |
| Risk-group assignment²           |       |       |       |
| Low                              | 0/5   | 0/2   | NS    |
| Intermediate                     | 0/5   | 1/2   |       |
| High                             | 5/5   | 1/2   |       |
| Extramedullary infiltration⁴     |       |       |       |
| Yes                              | 0/7   | 2/2   | 0.014 |
| No                               | 7/7   | 0/2   |       |
| Neurological symptoms⁴           |       |       |       |
| Yes                              | 0/5   | 0/2   | NS    |
| No                               | 5/5   | 2/2   |       |
| Treatment response               |       |       |       |
| Blast count on day 8 (blast µL⁻¹)² | 1564 (243-3800) | 41730 (20-83 440) | NS |
| Complete remission²             |       |       |       |
| Yes                              | 4/4   | 2/2   | NS    |
| No                               | 0/4   | 0/2   |       |
| Relapse²                         |       |       |       |
| Yes                              | 5/5   | 1/2   | NS    |
| No                               | 0/5   | 1/2   |       |
| Death²                           |       |       |       |
| 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.

² Prednisolone response, sample size for FCM+ = 2 and FCM− = 4.
³ Sample size FCM+ = 2 and FCM− = 5.
⁴ 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+            | p     |
|------------------------------------|-----------------|-----------------|-------|
| **At diagnosis**                   |                 |                 |       |
| White blood cells in peripheral blood (1 × 10^3 µL⁻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 (g dL⁻¹)               | 9 (6.1–13.7)    | 12 (9.1–17.1)  | NS    |
| Platelets (1 × 10^3 µL⁻¹)          | 60 (21–131)     | 92 (27–149)    | NS    |
| Neutrophils (1 × 10^3 µL⁻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 |
| **Cytogenetic findings**           |                 |                 |       |
| t(8:21)                            | 1/4             | 1/3             | NS    |
| GATA                               | 1/4             | 1/3             |       |
| Normal                             | 1/4             | 0/3             |       |
| Hyperdiploid                       | 1/4             | 1/3             |       |
| **Risk-group assignment**          |                 |                 |       |
| Favourable                         | 1/5             | 1/3             | NS    |
| Intermediate                       | 0/5             | 1/3             |       |
| Unfavourable                       | 4/5             | 1/3             |       |
| **Extramedullary infiltration**    |                 |                 |       |
| Yes                                | 1/5             | 3/3             | 0.04  |
| No                                 | 4/5             | 0/3             |       |
| **Neurological symptoms**          |                 |                 |       |
| Yes                                | 0/5             | 1/3             | NS    |
| No                                 | 5/5             | 2/3             |       |
| **Treatment response**             |                 |                 |       |
| 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.

a 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  |
|-----------------------------------------------|-----------------------|-------------------|
| White blood at diagnosis ($> 50000 \mu L^{-1}$) | 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           |

**Risk**

| Risk     | Relapse-free survival | Overall survival |
|----------|-----------------------|------------------|
| Low      | 0.07                  | 0.18             |
| Intermediate | 0.58                  | 0.20             |
| High     | 0.11                  | 0.14             |

**Cytogenetics**

| Cytogenetics | Relapse-free survival | Overall survival |
|--------------|-----------------------|------------------|
| 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.

![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) $p = 0.13$.](image)

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

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

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) $p = 0.28$.

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