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ORIGINAL ARTICLE

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

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## Abstract

Central nervous system infiltration by acute leukemia is a poor prognosis variable, and conventional cytology is the gold standard for its diagnosis; the technique is highly specific but not sensitive. To improve the diagnosis, flow cytometry has been used in different studies, showing greater sensitivity in the detection of leukemic cells. This study aimed to evaluate the presence of tumor cells by flow cytometry and conventional cytology, in cerebrospinal fluid from patients with acute leukemia as well as its relationship with clinical and biological parameters. In total, 156 CSF samples from 55 children with acute leukemia were studied. We found the following results: FCM−/CC− 131/156; FCM+/CC− 19/156; FCM−/CC+ 0; FCM+/CC+ 1/156; FCM−/CC suspicious 1/156; and FCM+/CC suspicious 4/156. Patients with B-cell acute lymphoblastic leukemia and FCM+ showed a lower response to steroid-treatment, abnormal karyotype, neurological symptoms, and worse relapse-free survival. Patients with T-cell acute lymphoblastic leukemia and FCM+ demonstrated association with thrombocytopenia. In conclusion, flow cytometry has greater sensitivity for the detection of tumor infiltration in cerebrospinal fluid, a finding that correlates with prognostic parameters in patients with acute leukemia.

**Keywords:** acute leukemia; CNS infiltration; flow cytometry; conventional cytology.

## Abbreviations key table

| Abbreviation | Full term |
|---|---|
| CNS | Central Nervous System |
| AL | Acute leukemia |
| ALL | Acute lymphoblastic leukemia |
| AML | Acute myeloid leukemia |
| CC | Conventional cytology |
| CSF | Cerebrospinal fluid |
| FCM | Flow cytometry |
| FCM+ | Infiltration in CNS detected by flow cytometry |
| FCM− | Infiltration in CNS not detected by flow cytometry |
| LDH | Lactate dehydrogenase |
| OS | Overall survival |
| RFS | Relapse-free survival |

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## 1. Introduction

Infiltration of the Central Nervous System (CNS) is associated with a worse prognosis in acute leukemia (AL) [1, 2]. Currently, conventional cytology (CC) of cerebrospinal fluid (CSF) is the gold standard test for the diagnosis of meningeal disease in cases of AL and other hematological neoplasms [3, 4]. However, despite its high specificity, CC has a limited sensitivity, with a false-negative rate from 20 % to 60 % [5, 6]. Recently, flow cytometry (FCM) analysis of CSF was found to be more specific and sensitive for the identification of infiltrating tumor cells, even when they are scarce [7, 8, 9]. The detection of tumor cells in the CSF has been associated with clinical and biological variables of poor prognosis and with a better risk group assignment of patients [10, 11]. These data show a clear advantage of FCM over CC for the detection of tumor cells in CSF. In particular, and according to laboratory guidelines from FCM experts, the quality of CSF must be improved with a cell stabilizing agent [12].

In Colombia, studies are needed to implement better (highly sensitive and specific) techniques for the early identification of tumor cells in the CNS. This way, a risk group assignment could be established timely to define a better therapeutic approach for improving overall and relapse-free survival of these patients. In this sense, our objective was to show the higher sensitivity of FCM in comparison with CC for the compulsory recommendation of FCM technique for the detection of tumor cells in the CSF in our patients. We compared the results of both techniques, considering parameters in pre-analytical and analytical phases, and we also demonstrate an association between FCM and clinical variables of prognostic impact in children with AL.

## 2. Methods

### 2.1. Patients and samples

Between 2008 and 2016, CSF samples from patients $< 18$ years of age with AL, assisted at the Hospital Universitario San Ignacio and Hospital Universitario Fundación Santa Fe de Bogotá, were collected. Patients were classified according to the risk classification described in the clinical practice guidelines from Colombia. CSF samples were obtained at diagnosis and during the follow-up after-treatment (on average, three samples per patient). All blood contaminated CSF samples were excluded from the study (traumatic lumbar punction). Parents or guardians signed a written informed consent approved by the Ethics Committees of both healthcare institutions. CSF samples were drawn by lumbar puncture and split into two equal aliquots. One aliquot was used for CC analysis and the other for FCM.

### 2.2. Cytological analysis

CC was carried out in the pathology laboratory of each institution, and the samples were examined by a cytopathologist. To meet the pre-analytical conditions of CC, CSF samples were kept at 4 °C and processed within the first hour of collection. Concentrated smears of CSF were prepared by Cytospin and stained with haematoxilyn-eosin and/or Giemsa. The finding of at least one immature (high nuclear to cytoplasm ratio and/or presence of nucleolus) cell per 100 fields observed under 100X oil immersion was considered as a positive result [4].

### 2.3. Immunophenotype

The FCM analysis of CSF was performed according to the protocols described by Kraan and Van Dongen *et al.* [13, 14]. We used multiparametric flow cytometry with 6 and 8 colors guided by the analysis recommendations of Euroflow's Infinicyt$^{TM}$ software (Cytognos S.L. Spain). In brief, at the moment of collection, the CSF sample was diluted 1/10 with Transfix$^{TM}$ (Cytomark) and kept at 4 °C in the dark for 18 hours until analysis [12]. **Supplementary material Table S1** shows the antibody panel used in accordance with the type of AL. For simultaneous staining of surface and intracellular antigens, fixation and permeabilization were performed according to the kit instructions (IntraStain – Dako, Denmark). To determine the absolute number of cells in the CSF samples, 50 µL fluorescent beads (CytoCount$^{TM}$ – DakoCytomation, Denmark) were added. Cells were acquired on a BD FACSCanto II flow cytometer (BD Biosciences).

### 2.4. Cell populations by FCM

Debris and artifacts were removed. The groupings of more than 25 events that shared the same forward scatter, side scatter and fluorescence intensity in each immunophenotypic marker analyzed were considered as cell populations. Clusters of less than 10 events were negative. In this case, cells in the CSF were characterized by their forward/scatter properties and fluorescence of lineage markers. Blasts were identified according to expression markers in the bone marrow.

If no tumor infiltration was detected after the analysis, the process was repeated with the same combination of antibodies to increase the assay sensitivity. Absolute cellular numbers by FCM were calculated according to the Current Protocols in Cytometry [13].

### 2.5. Analysis of laboratory and clinical data

For correlation analysis between clinical variables and FCM findings, patients were divided into two groups, those positive for infiltration detected by FCM (FCM+) and those negative for infiltration detected by FCM (FCM−).

Clinical data were collected to identify variables associated with higher relapse-risk or death in patients with CNS infiltration: age, gender, diagnosis, cytogenetics findings, peripheral blood leucocyte count at diagnosis, hemoglobin, platelets, lactate dehydrogenase (LDH), neutrophils, extramedullary infiltration, neurologic symptoms, 8-day post-treatment response, complete remission, relapse, and death. The hypothesis predicted a relationship between these variables and patients with FCM+.

### 2.6. Statistics

All statistical analyses were performed using SPSS for Windows (version 20; SPSS, Chicago, IL, USA). Agreement between the techniques was made using the Kappa index and $\chi^2$-test. Therefore, Mann-Whitney and Wilcoxon tests were used to compare quantitative variables. Overall survival (OS) and relapse-free survival (RFS) curves were constructed according to the Kaplan-Meier method. Survival curves were compared by the log-rank test. FCM+ and FCM− patients were compared according to their leukemia phenotype and risk group assignment. RFS was defined as the length of time since the primary treatment until the first event of a therapeutic failure when the disease returned. OS was determined by the condition of the patient, alive or dead, at the moment of reviewing clinical data. Death was considered if it was a death associated with cancer and no other causes.

Cox regression was made to determine if there was a relationship between OS and RFS, considering the variables of prognostic impact and infiltration in the CNS detected by FCM. According to the Wald test, results of the full models are shown with estimated hazards ratios, confidence interval of 95 %, and $p$-values. Results were considered statistically significant at $p < 0.05$.

## 3. Results

### 3.1. Samples and patients

In total, 55 patients were included in the study: 16 females (29 %) and 39 males (71 %). Infiltration of the CNS was analyzed by FCM and CC in all subgroups of patients. At diagnosis, FCM was positive in 3/37 (8 %) patients with B-cell acute lymphoblastic leukemia (B-cell ALL), 1/8 (12 %) with T-cell acute lymphoblastic leukemia (T-cell ALL), 1/8 (12 %) with acute myeloid leukemia (AML), and 1/2 (50 %) with mixed-lineage acute leukemia. During the follow-up, FCM+ results in cases of CNS relapse by acute lymphoblastic leukemia (ALL) were also detected (**Supplementary material Table S2**).

### 3.2. FCM and CC results

In total, 156 samples were collected for analysis by FCM and CC, and the results were as follows: 153 FCM−/CC−: 131 (84 %); FCM+/CC−: 19 (12.2 %); FCM−/CC+: 0 (0 %); FCM+/CC+: 1 (0.64 %); FCM−/CC suspicious: 1 (0.64 %); and FCM+/CC suspicious: 4 (2.56 %) (**Table 1**). It is important to highlight that the positive CC result was only observed when FCM identified a high percentage of blasts (98 %; 13.232 $\mu L^{-1}$) in the CSF. The agreement between CC and FCM showed a Kappa index of 0.17, indicating a poor concordance between the two techniques.

### 3.3. Cell populations detected by FCM

In this study, cell subsets were detected in sample volumes $\geq$ 1 mL. In addition to blasts, other cell subsets, such as monocytes, T-cells, B-cells, and neutrophils, were identified in CSF samples. Because most CSF samples corresponded to patients with B-cell ALL, the analysis of cell populations was only performed in this group of patients. It was found that FCM+ patients showed higher absolute counts of T-cells when compared to FCM- patients (**Supplementary material Table S3**).

**Table 1.** FCM and CC analysis of CSF for the detection of CNS Flow cytometry tumor infiltration in samples of patients with AL ($n = 156$)

| | Flow cytometry | | | |
|---|---|---|---|---|
| **Conventional cytology** | **Positive** | **Negative** | **Total** | $p^a$ |
| Positive | 1 | 0 | 1 | |
| Negative | 19 | 131 | 150 | **< 0.001** |
| Suspicious | 4 | 1 | 5 | |
| Total | 24 | 132 | 156 | |

[a] Chi-square test.

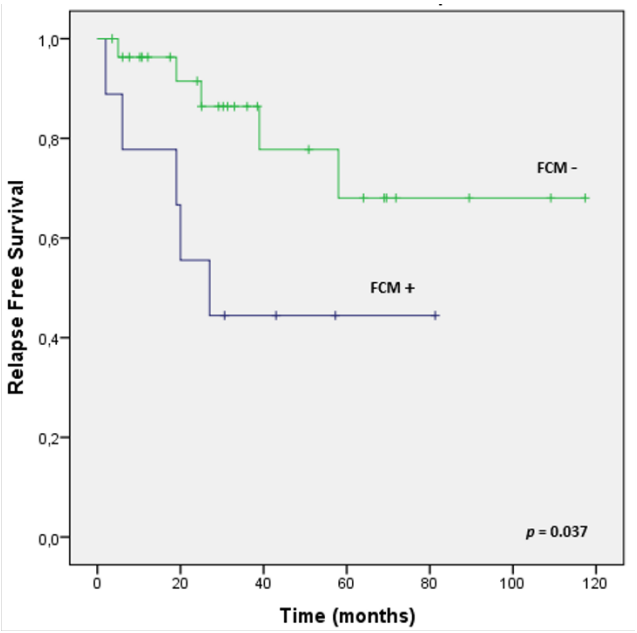
### 3.4. Infiltration detected by FCM and its association with clinical variables

In the group of patients with B-cell ALL, 10/37 were FCM+. The infiltration was associated with clinical factors, such as poor prognosis cytogenetics, risk group stratification, neurological symptoms, and poor response to corticosteroid treatment. They further showed an association with clinical outcomes, such as relapse and death (**Table 2**). The patients were stratified into 3 risk groups: low, intermediate, and high, considering age, white blood cell count, lineage, genetics, molecular and response to day 8, 15 and end of induction [15].

In the group of patients with T-cell ALL, 2/8 patients showed FCM+ results, a finding associated with an increasing trend in thrombocytopenia. There was no association between FCM+ and the clinical variables evaluated (**Supplementary material Table S4**). In the cases of patients with AML, 3/8 patients were FCM+, but there were no associations between FCM+ and the clinical variables analyzed (**Supplementary material Table S5**).

### 3.5. Survival analysis

Since most of our patients corresponded to the B-cell ALL phenotype, we decided to perform survival analyses only in the group of patients diagnosed with B-cell ALL, considering the variable CNS infiltration detected by FCM. The analysis of RFS demonstrated, like the result found in the group of patients with LA, that patients with FCM+ had a shorter RFS time than patients with FCM− (**Figure 1**), although the OS curves for both groups of patients were not significantly different (Supplementary material **Figure S1**).




| | Patients (n) | Relapse (n) | RFS (mean in months) |
|---|---|---|---|
| FCM + | 9 | 5 | 44 |
| FCM - | 28 | 5 | 91 |

**Figure 1.** Relapse-free survival of B-cell ALL patients with (blue line) or without (green line) CNS tumor infiltration detected by FCM. Log-rank test (Mantel-Cox) $p = 0.03$.

Finally, since most of the patients with B-cell ALL were classified as high risk, we decided to perform the outcome analyses only in this group to reach statistical significance. Patients with B-cell ALL and high-risk showed a tendency to relapse faster among patients with FCM+ than patients with FCM− (**Supplementary material Figure S2**), and there was no significant difference in OS in these groups (**Supplementary material Figure S3**).

**Table 2.** Prognosis factors in patients with B-cell ALL according to CNS tumor infiltration detected by FCM analysis of CSF.

| FACTOR | | FCM− | FCM+ | *p* |
|---|---|---|---|---|
| **At diagnosis**[a] | | | | |
| White blood cells in peripheral blood ($1 \times 10^3\ \mu L^{-1}$) | | 19 (2-161) | 63.4 (4.2-426) | NS |
| Blasts in bone marrow (%) | | 81.5 (30-97) | 63 (24-95) | NS |
| Platelets ($1 \times 10^3\ \mu L^{-1}$) | | 111 (6.3-417) | 88 (6-287) | NS |
| Neutrophils ($1 \times 10^3\ \mu L^{-1}$) | | 3.2 (0.1-28) | 14.1 (0.06-101.4) | NS |
| Blasts in peripheral blood (%) | | 34.5 (0-89) | 38 (0-95) | NS |
| Hemoglobin ($g\ dL^{-1}$) | | 9.1 (5.1-14.5) | 8.3 (4.6-16.7) | NS |
| LDH (UI/L) | | 1679 (367-6 263) | 1122 (350-2627) | NS |
| **Cytogenetic findings** | | | | |
| | $t(9;22)$ | 2/25 | 3/9 | |
| | Normal | 16/25 | 5/9 | |
| | $t(12;21)$ | 0/25 | 1/9 | **0.033** |
| | $t(1;19)$ | 2/25 | | |
| | Hyperdiploid | 5/25 | | |
| **Risk-group assignment** | | | | |
| | Low | 11/26 | 0/10 | |
| | Intermediate | 5/26 | 1/10 | **0.005** |
| | High | 10/26 | 9/10 | |
| **Extramedullary infiltration** | | | | |
| | Yes | 1/26 | 10/10 | **< 0.001** |
| | No | 25/26 | | |
| **Neurological symptoms** | | | | |
| | Yes | 0/26 | 6/9 | **< 0.001** |
| | No | 26/26 | 3/9 | |
| **Treatment response** | | | | |
| Blast count on day 8 ($blast\ \mu L^{-1}$)[b] | | 206 (0-2,205) | 47 782 (0-282,768) | **0.024** |
| **Complete remission** | | | | |
| | Yes | 25/26 | 9/10 | NS |
| | No | 1/26 | 1/10 | |
| **Relapse** | | | | |
| | Yes | 5/25 | 6/10 | **0.023** |
| | No | 20/25 | 4/10 | |
| **Death** | | | | |
| | Yes | 3/26 | 5/10 | **0.014** |
| | No | 23/26 | 5/10 | |

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+ = 8 and FCM− = 26.

[b] Prednisolone response, sample size for FCM+ = 8 and FCM− = 19.

We considered that many other factors may affect the clinical outcomes of patients; for this reason, we conducted a multivariate analysis to determine which variables of prognostic impact could be determining the survival of patients, including the CNS detected by FCM. First, among all patients with LA, we found that the variables that contribute to the model for both RFS and OS are infiltration detected by FCM, complete remission and $t(9;22)$ (**Supplementary material Table S6**).

Subsequently, we performed the same analysis with only patients with B-cell ALL, considering the same variables and adding the response to prednisolone. In this analysis, we found that the variables that contribute to the event for RFS are: complete remission, infiltration to the CNS, $t(9;22)$ and $t(4;11)$. The statistically significant variables that affected OS are: white blood cell count at diagnosis, complete remission, CNS infiltration detected by FCM, response to prednisolone, risk classification and the $t(9;22)$ (**Table 3**).

## 4. Discussion

In recent years, analysis of CSF by FCM has been implemented as an alternative tool for the diagnosis of CNS tumor infiltration in patients with lymphoma and AL, where FCM has shown better performance than CC [7, 11, 16, 17].

Recently, Popov *et al.* compared the results from FCM and CC in 155 pediatric patients with ALL, and they found that FCM was positive in 35.3 % of the patients, while CC was positive in only 15.3 %. The difference between the FCM and CC is almost double compared to the cases analyzed, and this result is very similar to that reported by us [18].

Similar results were found by Gong *et al.* who conducted a study in patients with ALL and found that 15 samples of CSF were positive using both techniques (FCM/CC), while 26 samples were positive using only FCM. This showed that FCM has higher sensitivity than CC for the diagnosis of adult patients with ALL and CNS infiltration [19]. Another study by Del Principe in patients

**Table 3.** Multivariate Cox model for relapse-free survival and overall survival of patients with B-cell ALL.

| | **Relapse-free survival** | **Overall survival** |
|---|---|---|
| **Variable** | ***p*** | ***p*** |
| White blood at diagnosis ($> 50\,000\,\mu L^{-1}$) | 0.12 | 0.04 |
| Complete remission | 0.006 | 0.003 |
| Age | 0.29 | 0.20 |
| CNS Infiltration by FCM | 0.01 | 0.04 |
| Prednisolone response | 0.09 | 0.03 |
| Risk | 0.07 | 0.04 |
| **Cytogenetics** | | |
| $t(9;22)$ | $< 0.001$ | $< 0.001$ |
| $t(4;11)$ | 0.17 | 0.57 |
| $t(12;21)$ | 0.42 | 0.57 |
| $t(1;19)$ | 0.30 | 0.20 |

B-cell ALL Patients with known data on all covariates in the model, relapse-free survival ($n = 8$) and overall survival ($n = 5$). Abbreviations: CNS: central nervous system. FCM: flow cytometry.

with AML found that 33 % were FCM+/CC+, while analysis using FCM alone detected 67 %, suggesting that FCM should be performed routinely because many cases go undetected with CC [20].

Among the 156 CSF samples analyzed for tumor infiltration, 19 (12.2 %) were FCM+ and only 1 (0.64 %) CC+; it is important to note that there were no FCM−/CC+ cases. A CC+ result was only observed when the FCM reported high levels of blasts (98 %, corresponding to 13 232 cell $L^{-1}$); i.e., when the patient already had many tumor cells in the CNS, in this case, without neurological symptoms of infiltration.

The present results evidenced that 6/9 patients with B-cell ALL and FCM+ had neurological symptoms, whereas they were free of disease according to CC. Gong *et al.* found similar results among patients who were FCM+/CC− (93.3 %), and even this result was higher than that evidenced in FCM+/CC+ patients (53.8 %) [19]. In this scenario, the advantage of FCM over CC is highlighted because it demonstrates an association between a positive result by FCM and signs of infiltration in the CNS.

On the contrary, in the present study, there were no discordant FCM−/CC+ cases, as has been reported by other authors [20, 21, 22]. We consider that, unlike the studies in which discordant results were found between CC and FCM, our study considered pre-analytical variables that could affect the performance of the techniques. The main difference between those studies and ours is the use of a CSF stabilizer, because this reactive stabilizes and preserves the CSF until its analysis by FCM [12]. This is advantageous for clinical diagnosis services that cannot process CSF in less than 1 hour after collection or that do not have the technology for their analysis and must send the sample to another institution. However, we believe that the size of the sample could have influenced this result and that it would be necessary to collect more samples to verify the absence of FCM−/CC+ results.

FCM allows the analysis of several cellular parameters, such as size, complexity and immunophenotypic markers, which can differentiate a tumor cell from a normal cell with greater accuracy. The above is difficult to achieve using CC because it only has morphological parameters that do not always allow the correct differentiation between healthy versus tumor conditions [23]. However, there were 4 FCM+/CC suspicious CSF from patients with neurological symptoms, a finding that supports the reliability of the FCM results. In addition, 1 FCM−/CC suspicious CSF was found in a patient without symptoms of CNS infiltration, supporting once more the results by FCM.

CC has several drawbacks that affect its performance: 1) it requires at least 10 mL of sample volume, 2) it requires several samples, 3) it needs to be analyzed quickly to avoid the loss of cell viability, and 4) differentiation between normal and neoplastic cells can be misleading [4, 23]. Given the previous disadvantages of CC, a standardized protocol was implemented by the EuroFlow consortium to overcome those drawbacks [14]. Since CSF suffers rapid deterioration of its content, including the cells inside it, CSF samples for FCM must be stabilized with a stabilizer agent and analyzed with 6-8 fluorescent markers to increase the sensitivity of the technique. Despite the multiple advantages of flow cytometry for the evaluation of special samples such as CSF, some disadvantages are also described that are directly related to low cell viability, loss of cells by centrifugation, contamination of samples with blood and analysis of samples [24, 25], the minimum number of events that should be considered as a cell population (groups of more than 25 events as positive, 10-25 events as suspect, and less than 10 events as negative) [13]. Our study showed that, by following the recommendations of the EuroFlow protocol [14] and using a

CSF stabilizer, blasts can be detected by FCM in volumes less than 1 mL (0.07 blasts $\mu L^{-1}$); the ability to detect blasts in small volumes of CSF is considered very important because it is not always feasible to extract a volume of 10 mL from a child.

It is also important to note that the blast detection by FCM was not conditioned to the repetition of lumbar punctures; in contrast, FCM was able to detect tumor cells in the first sample taken from 6 patients with AL (11 %). These samples corresponded to the sample taken at the time of diagnosis and provided data on the Colombian incidence of CNS infiltration detected by FCM at the time of diagnosis in pediatric patients with AL, which was unknown until now. The samples of these 6 patients were negative by CC. The problem with these findings is that CC (the gold standard) is not detecting patients with infiltration in the CNS upon diagnosis, and this can generate an inadequate risk classification.

Even though differentiation between normal and neoplastic cells can be misleading with both techniques because both are dependent operator, FCM allows the analysis of several cellular parameters, such as size, complexity and immunophenotypic markers, which can differentiate a tumor cell from a normal cell with greater accuracy. Comparison of cell subsets between FCM+ and FCM− CSF samples showed a significant difference in patients with B-cell ALL; in fact, higher numbers of T-cells were found in FCM+ patients than FCM- patients. In healthy conditions, the blood-brain barrier allows the passage of small amounts of leucocytes, mainly lymphocytes and monocytes [24, 25, 26]. It has been described that the proinflammatory environment in cancer favors the migration of tumor cells to other anatomical sites [27, 28]. Under this context, patients with FCM+ may have a proinflammatory environment as evidenced by the increase of lymphocytes, and this abnormal increase of leucocytes in CSF could be favoring the passage of tumor cells to the SNC. Nevertheless, we do not know the lymphocyte subpopulations present in the CSF.

Regarding the association between FCM and clinical variables of prognostic impact, we find that, CNS tumor infiltration was associated with thrombocytopenia inpatients with T-cell ALL. Thrombocytopenia has been related to a higher risk of CNS metastases because it increases the risk of bleeding at the moment of lumbar puncture, favoring the entry of blasts into the CNS [1, 27]. In patients with AML, no association was found between the infiltration detected by FCM and clinical variables of prognostic impact. Larger samples of patients with T-cell ALL and AML phenotypes, as well as longer follow-ups, would be necessary to precisely evaluate those clinical relationships.

In patients with B-cell ALL, the $t(9;22)$ was detected in 33 % of patients with FCM+ and only in 8 % of patients with FCM−. The $t(9;22)$ (q34; q11) with BCR-ABL, $t(4;11)$ with MLL-AF4 inv 16, and $t(1;19)$ with E2A-PBX1 have been described in association with a higher risk of CNS tumor infiltration; these anomalies, together with other factors, impact the risk-group assignment of patients [1, 2].

Patients with B-cell ALL and FCM+ CSF showed a lower prednisolone response and major relapse and death, and these results suggest that CNS infiltration directly impacts the clinical outcomes of these patients. Additionally, they relapsed faster compared with patients without leptomeningeal compromise, as observed in the RFS curves. These findings may support the concept that the CNS acts as a sanctuary site for leukemic blasts because it has been shown that systemic chemotherapy may induce the migration of tumor blasts to anatomical sites that block the entry of chemotherapy, creating a special niche that favors the survival of these cells that could later cause patient relapse [29, 30, 31].

Finally, our analysis of survival and multivariate Cox models shows a clear relationship between having CNS infiltration and presenting relapse or death. These data were observed in groups of patients with AL and B-cell ALL. Multivariate analysis allows us to identify which variables favor clinical outcomes in an independent form. These results indicate that the infiltration detected by FCM, as well as other variables of prognostic impact, favor relapse and/or death of patients. It is not possible to obtain valid conclusions in the group of patients with T-cell ALL and AML, because we do not have an adequate number of patients to find significant differences. Del Principe *et al.* and Martinez-Laperche *et al.* found similar results in terms of a lower survival of patients with FCM+/CC as well as CNS infiltration as independent variables that affect the survival of patients [7, 8].

In conclusion, the results suggest that pediatric patients with AL will benefit from the implementation of FCM for diagnosis of CNS tumor infiltration, even in the absence of neurological symptoms. CSF analysis by FCM can detect minimal numbers of blasts that could be associated with CNS occult disease. Compared to CC, FCM has the advantage of being a more convenient diagnosis tool because it can detect an occult leptomeningeal compromise, directly improving the proper risk classification and prognosis of patients.

## 5. Conflict of Interest Statement

Nothing to declare.

## 6. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## 7. Acknowledgments

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## Utilidad de la citometría de flujo en la detección de células tumorales en fluido cerebroespinalde pacientes con leucemia aguda.

**Resumen:** La infiltración del sistema nervioso central por leucemia aguda es una variable de prognosis pobre, y la citología convencional es el estándar por excelencia en su diagnóstico; la técnica es altamente específica, pero no sensible. Para mejorar el diagnóstico, la citometría de flujo se ha usado en diferentes estudios, y ha mostrado mayor sensibilidad en la detección de células leucémicas. En el presente estudio se evaluó la presencia de células tumorales por citometría de flujo y por citología convencional en fluido cerebroespinal de pacientes con leucemia aguda, así como su relación con parámetros clínicos y biológicos. En total se estudiaron 156 muestras de FCE de 55 niños con leucemia aguda. Se encontraron los siguientes resultados: CMF−/CC− 131/156; CMF+/CC− 19/156; CMF−/CC+ 0; CMF+/CC+ 1/156; CMF−/CC sospechoso 1/156; y CMF+/CC sospechoso 4/156. Los pacientes con leucemia linfoblástica aguda de células B y CMF+ mostraron una menor respuesta al tratamiento con esteroides, cariotipo anormal, síntomas neurológicos y una menor sobrevida libre de recaída. Los pacientes con leucemia linfoblástica aguda de células T y CMF+ demostraron asociación con trombocitopenia. En conclusión, la citometría de flujo tiene una mayor sensibilidad para la detección de infiltración de tumores en fluido cerebroespinal, un hallazgo que se correlaciona con los parámetros de prognosis en pacientes con leucemia aguda.

**Palabras Clave:** leucemia aguda; infiltración SNC; citometría de flujo; citometría convencional.

## A utilidade da citometria de fluxo na detecção de células tumorais no líquido cefalorraquidiano de pacientes com leucemia aguda

**Resumo:** A infiltração do sistema nervoso central por leucemia aguda é uma variável associada a mau prognóstico e o padrão ouro para diagnóstico é a citologia convencional; esta técnica é altamente específica, mas não é muito sensível. Para melhorar o diagnóstico, diferentes estudos têm usado citometria de fluxo, mostrando uma maior sensibilidade na detecção de células leucêmicas. O objetivo deste estudo foi avaliar a presencia de células tumorais por citometria de fluxo e citologia convencional, no líquido cefalorraquidiano de pacientes com leucemia aguda, assim como sua relação com parâmetros biológicos e clínicos. No total foram estudadas 156 amostras de líquido cefalorraquidiano provenientes de 55 crianças. Obtivemos os seguintes resultados: FCM−/CC− 131/156; FCM+/CC− 19/156; FCM−/CC+ 0; FCM+/CC+ 1/156; FCM−/CC suspeito 1/156; e FCM+/CC suspeito 4/156. Pacientes com Leucemia linfoblástica aguda de células B e FCM+ tiveram uma menor resposta a tratamento com esteroides, cariótipos anormais, sintomas neurológicos e pior sobrevivência livre de recaídas. Pacientes com Leucemia linfoblástica aguda de células T e FCM+ mostraram uma associação com trombocitopenia. Em conclusão, a citometria de fluxo é mais sensível que a citologia convencional na detecção de infiltração tumoral no líquido cefalorraquidiano, este resultado está correlacionado com parâmetros prognósticos em pacientes com leucemia aguda.

**Palavras-chave:** leucemia aguda; infiltração do sistema nervosa central; citometria de fluxo; citologia convencional.

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