Flow cytometric analysis of cerebrospinal fluid identifies patients with occult central nervous system involvement in relapsed pediatric acute lymphoblastic leukemia

Central nervous system (CNS) involvement is associated with a poor prognosis and higher risk of CNS relapse in childhood acute lymphoblastic leukemia (ALL).[1] The conventional method for identifying CNS involvement is by cytospin of cerebrospinal fluid (CSF) and microscopic examination. This method is challenged by low sensitivity and specificity for rare cells and can give rise to both false-negative and -positive results.[2] Several studies now support that flow cytometry (FCM) is more sensitive in detecting leukemic blasts in CSF.[7,8] FCM identifies leukemic blasts based on aberrant expression of cellular markers and is highly specific in detecting malignant cells.[2,7] At initial diagnosis, CNS involvement by FCM has been shown to be an independent prognostic factor for relapse.[8-11] Reports also show that FCM can be used for monitoring CNS disease during treatment[8,10,12,13] and ongoing clinical studies are investigating whether the clearance of leukemic blasts in the CSF during treatment holds prognostic information, similarly to minimal residual disease (MRD) monitoring in the bone marrow (BM).

Several studies have reported CSF FCM results from the time of diagnosis, but only two small studies have examined CNS involvement by FCM at the time of relapse.[13,14] We here present CSF FCM results for 39 patients with relapsed ALL of any site to investigate the diagnostic value of CSF FCM at relapse.

Patients aged 0-17.9 years at initial diagnosis of ALL were enrolled in a prospective CSF FCM study at 17 hospitals in Denmark, Finland, Lithuania, Norway, and Sweden from September 2012 to December 2022. The study was conducted in accordance with the Declaration of Helsinki and approved by the national ethical committees. Written consent was obtained from legal guardians of all participants. CSF samples were collected into Transfix® tubes (Intermedico Ltd., Hellerup, Denmark) at the first lumbar puncture and until treatment day 15 after relapse. Samples were shipped to the National Danish MRD laboratory, Rigshospitalet, Copenhagen, Denmark for FCM analysis as previously described.[10] The samples were analyzed using standardized FCM panels from either the NOPHO2008 protocol (BCP: CD3/CD34/CD19/CD10/CD38/CD20/CD45; T-ALL: CD3/CD4/CD7/CD8/CD45/CD56) or the ALLTogether1 protocol (BCP: CD81/CD3/CD34/CD19/CD10/CD38/CD20/CD45; T-ALL: HLA-DR/CD7/CD3/CD14/CD33/CD8/CD4/CD45/CD56+CD16). The minimal number of aberrant cells required to define CNS positivity was ten BCP or T cells with a leukemia-associated phenotype (LAIP). In difficult cases information about aberrant marker expression by BM blasts was requested to identify the blasts. Blast count in CSF (blasts/mL) was determined as blasts with a LAIP detected by FCM divided by the volume of CSF minus 200 μL (volume of TransFix per tube). Operators examining FCM or cytospin samples were blinded to the results from the other method. FCM results were blinded to the treating physician. Statistical analysis was performed with R version 4.3.0.

We collected a total of 63 CSF samples (median volume: 2.15 mL; interquartile range [IQR], 1.8-2.3; median time to FCM analysis: 1 day; IQR, 0-2 days) from 39 patients with ALL at the time of first relapse (Table 1). The cohort included patients with isolated BM relapses (N=14), CNS-involving relapses (N=19) and relapses involving other sites (N=6). Eight patients were diagnosed with T-ALL and 31 patients with BCP-ALL. Three patients were infants (<366 days of age) at initial diagnosis, and two patients were diagnosed with Philadelphia chromosome-positive (Ph^+) ALL. At initial diagnosis, 32 patients were treated according to NOPHO2008 (EudraCT 2008-003235-20), three patients according to ALLTogether1 (EudraCT 2018-001795-38), two patients according to Interfant-06 (EudraCT 2005-004599-19) and two patients according to EsPhALL2010 (EudraCT 2004-001647-30). At relapse, 29 of 39 patients were treated according to IntReALL2010 (EudraCT 2012-000793-30/2012-000810-12). The remaining ten patients were treated with a combination of chemotherapy, irradiation, hematopoietic stem cell transplantation, Carfilzomib and immunotherapy.

For 24 (61.5%) of the 39 patients, leukemic blasts were detected by FCM in the initial CSF sample at relapse (Table 2). The median blast count for the 24 FCM positive samples was 667 blasts/mL (IQR, 32-66,341 blasts/mL). Nineteen (48.7%) relapses were classified as CNS-involving based on cytospin results, and all cytospin-positive patients were also positive by FCM. No relapses were classified as CNS-involving based on neurological symptoms and/or MRI results only. The five relapses that were CNS positive only by FCM included three morphological BM relapses, one relapse in the BM and testis, and one relapse in the BM, mediastinum, and abdomen. The median blast count for CSF samples positive by both FCM and cytospin was 20,679 blasts/mL (IOR, 112-112,714 blasts/mL) compared to 26 blasts/mL (IQR, 13-77 blasts/mL) for CSF samples positive only by FCM (P=0.012).

At primary diagnosis, all patients except six were classified as CNS1 based on cytospin results (Table 1). For 18 patients, we had CSF FCM data at both primary diagnosis and relapse. Eight (44.4%) patients were CNS positive by FCM at primary diagnosis, but only two patients were also cytospin positive.

Table 1. Clinical characteristics of 39 included patients at diagnosis and relapse.

Patient ID	Age at dx, years	Sex	Immuno-phenotype	WBC at dx, x10^9/L	CNS status at dx	Cytogenetic features	CSF FCM at dx, blasts/mL (total mL)	Treatment protocol at dx	Relapse site	Time to relapse, years	CSF FCM at relapse, blasts/mL (sample vol., mL)	Treatment protocol at relapse and outcome
1	12	M	BCP-ALL	6.7	CNS1	-	-	NOPHO2008	BM	3.8	0 (1.8)	IntReALL SR 2010; CR2, 2nd relapse in BM
2	13	F	BCP-ALL	2.7	CNS1	Hypodiploid	-	NOPHO2008	BM	1.7	0 (1.8)	NA; death <2 months after relapse
3	1	M	BCP-ALL	8.5	CNS1	ETV6/RUNX1	-	NOPHO2008	BM	2.7	0 (1.8)	NOPHO2008 blocks + HSCT; CR2
4	9 months	F	BCP-ALL	11.1	CNS1	TCF3/PBX1	0 (2.2)	Interfant-06	BM	3.0	0 (1.8)	IntReALL SR 2010; CR2
5	7	F	BCP-ALL	0.7	CNS1	HeH	-	NOPHO2008	BM	3.8	0 (2.0)	IntReALL SR 2010; CR2
6	12	M	T-ALL	18.6	CNS1	-	-	NOPHO2008	BM	9 months	0 (2.0)	IntReALL SR 2010; death <4 months after relapse
7	4	M	BCP-ALL	68.0	CNS1	-	35 (3.8)	NOPHO2008	BM+testis	2.8	0 (2.0)	IntReALL SR 2010 + HSCT; CR2
8	2	M	BCP-ALL	18.4	CNS1	-	-	NOPHO2008	BM+other site(s)	4.1	0 (2.0)	IntReALL SR 2010; CR2, 2nd relapse in testis
9	8	M	BCP-ALL	388.0	CNS1	-	8 (2.2)	NOPHO2008	Testis	3.8	0 (2.2)	IntReALL SR 2010; CR2, 2nd relapse in BM + CNS
10	5	M	BCP-ALL	6.8	CNS1	ETV6/RUNX1	-	NOPHO2008	BM	6.9	0 (2.3)	IntReALL SR 2010; CR2
11	11	F	BCP-ALL	149.0	CNS1	-	0 (2.8)	A2G-1 pilot	BM	6 months	0 (2.3)	Carfilzomib trial + IntReALL HR induction + inotuzumab + blinatumumab; death <4 months after relapse
12	17	M	BCP-ALL	8.3	CNS1	HeH	0 (2.3)	NOPHO2008	Testis	2.7	0 (2.3)	Irradiation + NOPHO2008 blocks + HSCT; CR2
13	14	F	T-ALL	14.9	CNS1	-	0 (2.8)	NOPHO2008	BM	10 months	0 (2.8)	NA; death <2 months after relapse
14	1	M	BCP-ALL	17.0	CNS1	-	-	NOPHO2008	BM+testis	3.2	13 (1.6)	IntReALL SR 2010; CR2, 2nd relapse in testis
15	4	M	T-ALL	11.6	CNS1	-	-	A2G-1 main protocol	BM+CNS	1.8	18 (2.3)	Isatuximab + topotecan, daunorubicin & trametinib + NOPHO2008 blocks; death <4 months after relapse
16	2	M	BCP-ALL	51.1	CNS1	-	0 (3.8)	NOPHO2008	BM	3.9	26 (1.8)	IntReALL SR 2010; CR2
17	14	M	BCP-ALL	86.0	CNS1	-	0 (2.1)	NOPHO2008	BM+CNS+testis	2.6	29 (1.8)	IntReALL HR 2010; CR2
18	3	F	BCP-ALL	81.1	CNS1	HeH	127 (1.8)	NOPHO2008	CNS	2.7	34 (0.8)	IntReALL SR 2010 + CNS irradiation; CR2, 2nd relapse in CNS
19	2	M	BCP-ALL	45.9	CNS1	-	1 (1.8)	NOPHO2008	BM+CNS	2.1	50 (0.3)	IntReALL SR 2010; CR2

Continued on following page.

Patient ID	Age at dx, years	Sex	Immuno-phenotype	WBC at dx, x10^9/L	CNS status at dx	Cytogenetic features	CSF FCM at dx, blasts/mL (total mL)	Treatment protocol at dx	Relapse site	Time to relapse, years	CSF FCM at relapse, blasts/mL (sample vol., mL)	Treatment protocol at relapse and outcome
20	9	F	BCP-ALL	47.3	CNS1	ETV6/RUNX1	-	NOPHO2008	CNS	2.2	297 (1.7)	IntReALL SR 2010 + HSCT; CR2
21	11 months	M	BCP-ALL	6.3	CNS1	ETV6/RUNX1, HeH	-	NOPHO2008	BM+CNS	5.8	667 (2.2)	IntReALL SR 2010; CR2
22	4	M	BCP-ALL	69.1	CNS1	-	-	NOPHO2008	BM+CNS	2.9	4,603 (2.3)	IntReALL HR 2010 + blinatumumab + HSCT; CR2
23	12	M	T-ALL	109.3	CNS1	-	7 (1.8)	NOPHO2008	CNS	10 months	12,992 (2.3)	IntReALL HR 2010 + CNS irradiation + HSCT; CR2, 2nd relapse in BM + CNS
24	2	M	BCP-ALL	17.2	CNS1	HeH	-	NOPHO2008	CNS	2.8	28,366 (1.8)	IntReALL SR 2010 + HSCT; CR2
25	2	M	T-ALL	66.2	CNS1	-	1,380 (4.2)	NOPHO2008	BM+CNS	9 months	34,476 (4.8)	IntReALL SR 2010 + HSCT; CR2, 2nd relapse in CNS
26	6	F	BCP-ALL	29.0	CNS1	BCR/ABL1	-	EsPhALL2010	BM+CNS	1.4	49,451 (2.0)	IntReALL SR 2010 + dasatinib + HSCT; CR2
27	8	M	BCP-ALL	1.1	CNS1	-	-	NOPHO2008	CNS	2.0	83,231 (2.0)	IntReALL SR 2010 + HSCT + NOPHO2008 blocks; CR2
28	9	F	BCP-ALL	1.6	CNS1	-	-	NOPHO2008	BM+CNS	3.4	122,541 (1.6)	IntReALL SR 2010; CR2, 2nd relapse in CNS
29	3	M	BCP-ALL	10.3	CNS1	ETV6/RUNX1		NOPHO2008	CNS	6.7	326,352 (1.3)	IntReALL SR 2010; CR2
30	5	M	BCP-ALL	156.1	CNS1	-	19 (2.4)	NOPHO2008	BM+CNS	2.7	348,229 (2.3)	IntReALL SR 2010; death <2 months after relapse
31	14	F	BCP-ALL	10.0	CNS1	HeH	0 (2.0)	NOPHO2008	CNS	2.6	395,387 (2.3)	IntReALL SR 2010 + HSCT; CR2
32	2	F	BCP-ALL	40.9	CNS1	HeH	-	NOPHO2008	BM+CNS	2.8	456,125 (2.2)	IntReALL SR 2010; CR2
33	3	M	BCP-ALL	4.8	CNS2	-	-	A2G-1 pilot	BM	1.5	0 (1.8)	Isatuximab + blinatumomab + HSCT; CR2
34	1	M	T-ALL	316.0	CNS2	-	0 (1.8)	NOPHO2008	BM+other site(s)	5.0	10 (2.1)	IntReALL HR 2010; CR2
35	9	M	T-ALL	4.3	CNS2	-	-	NOPHO2008	BM+CNS	1.4	21 (4.3)	Isatuximab + NOPHO2008 blocks + HSCT; CR2
36	15	M	BCP-ALL	3.3	CNS3	ETV6/RUNX1	0 (1.9)	NOPHO2008	BM	2.5	0 (1.8)	IntReALL SR 2010; CR2, 2nd relapse in BM
37	14	M	BCP-ALL	220.0	CNS3	BCR/ABL1	10,727 (2.2)	EsPhALL2010	CNS	1.7	100 (NA)	IntReALL HR 2010 + dasatinib + HSCT; CR2
38	2	M	T-ALL	141.0	CNS3	-	1,724 (2.2)	NOPHO2008	BM	7.2	121 (0.8)	Carfilzomib trial + HSCT; CR2
39	2 months	M	BCP-ALL	759.0	NA	KMT2A-r	-	Interfant-06	BM	7 months	77 (2.3)	6MP + CART; CR2

Continued on following page.

Patients are listed according to site of relapse and flow cytometry (FCM) status at relapse. Cerebrospinal fluid (CSF) FCM results are shown as blasts/mL of CSF and with volume of analyzed CSF in parentheses. Age and time from the primary diagnosis to relapse is shown in months if <1 year. dx: diagnosis; vol: volume; WBC: white blood cell count; CNS: central nervous system; CSF: cerebrospinal fluid; M: male; F: female; BCP-ALL: B-cell precursor acute lymphoblastic leukemia; T-ALL: T-cell acute lymphoblastic leukemia; HeH: high hyperdiploid; A2G: ALLTogether; BM: bone marrow; NA: not available; SR: standard risk; HR: high risk; CR2: second complete remission; HSCT: hematopoietic stem cell transplantation.

At relapse, 13 of 18 patients were positive by FCM. For the six patients FCM positive at both initial diagnosis and relapse, there was no significant correlation between blast levels (rS=-0.3; *P*=0.68) (*Online Supplementary Figure S1*).

We received one or more CSF samples during the first 2 weeks of treatment from 12 of 24 FCM positive patients at relapse (Figure 1). All samples were still FCM positive at day 4 (N=5/5), 80% (N=8/10) were positive at day 8, and 85.7% (N=6/7) were positive at day 15. In comparison, only 42% (N=5/12) of the patients had a follow-up sample positive by cytospin. Among the patients remaining positive by FCM at day 15 were two Ph^+ patients and two high hyperdiploid patients. Large blast count variation was seen between patients, and fluctuation in the blast levels were observed, since we did not receive samples from all patients at all time points (day 0: 28,366 blasts/mL; IQR, 2,635-66,341; day 4: 6,900 blasts/mL; IQR: 11-11,474; day 8: 36 blasts/mL; IQR, 23-270; day 15: 84 blasts/mL; IQR, 42-91) (Figure 1). Overall, the median blast count did however decrease from day 0 to day 15.

In the present study, we showed that 13% of the included relapses were classified as CNS positive only by CSF FCM and not by cytospin. The median blast level for CSF samples with blasts on both cytospin and FCM was about 1,000-fold higher than samples only positive by FCM. The median blast level in CSF determined by FCM was 25-fold higher at relapse than at initial diagnosis in our previously published study.[10] This shows that CSF FCM identifies a subset of patients with occult CNS disease at relapse, despite the high general blast load in the CNS at relapse. This is supported by a previous CSF FCM study that analyzed samples from 13 children with isolated BM relapse, and at the first lumbar puncture 54% of these patients had blasts in their CSF by FCM.[13]

For CSF samples collected during treatment from relapse patients who were FCM positive at the first lumbar puncture, 80-90% of CSF samples remained positive throughout the first 15 days of treatment. The CSF is thus not cleared as rapidly of blasts at relapse as at initial diagnosis, where less than 10% of the samples remained positive at day 15.[10] This could imply that the persistent leukemic cells at relapse are a selected subpopulation, or that they reside in a protective CNS niche. Cytospin samples collected after the initial lumbar puncture were only positive in about half of the patients, which is consistent with the significant reduction in blast levels during treatment. Even though our results and a previous study[13] demonstrate higher sensitivity of FCM in detecting blasts in CSF during relapse treatment, the prognostic impact of this remains undetermined.

The current study covers the largest published cohort of pediatric ALL patients with CSF flow data from the time of relapse. A strength of the study is that FCM was performed in a MRD flow laboratory with staff very experienced in analyzing CSF samples. The drawbacks are that the cytospin samples were not centrally reviewed, and that follow-up samples for CSF FCM only were available in 50% of the patients with FCM-positive results at relapse. In addition, the total number of patients was too small to examine the

Table 2. Central nervous system classification of 39 patients by flow cytometry and cytospin at first relapse.

Cases by FCM	Cases by cytospin		
	CNS neg, N (%)	CNS pos, N (%)	Total, N (%)
CNS neg	15 (38.5)	0 (0)	15 (38.5)
CNS pos	5 (12.8)	19 (48.7)	24 (61.5)
Total	20 (51.3)	19 (48.7)	39 (100)

Central nervous system (CNS) positivity by flow cytometry (FCM) is defined as ≥ 10 blasts with a leukemia-associated phenotype. CNS positivity by cytospin corresponds to CNS2/CNS3. neg: negative; pos: positive.

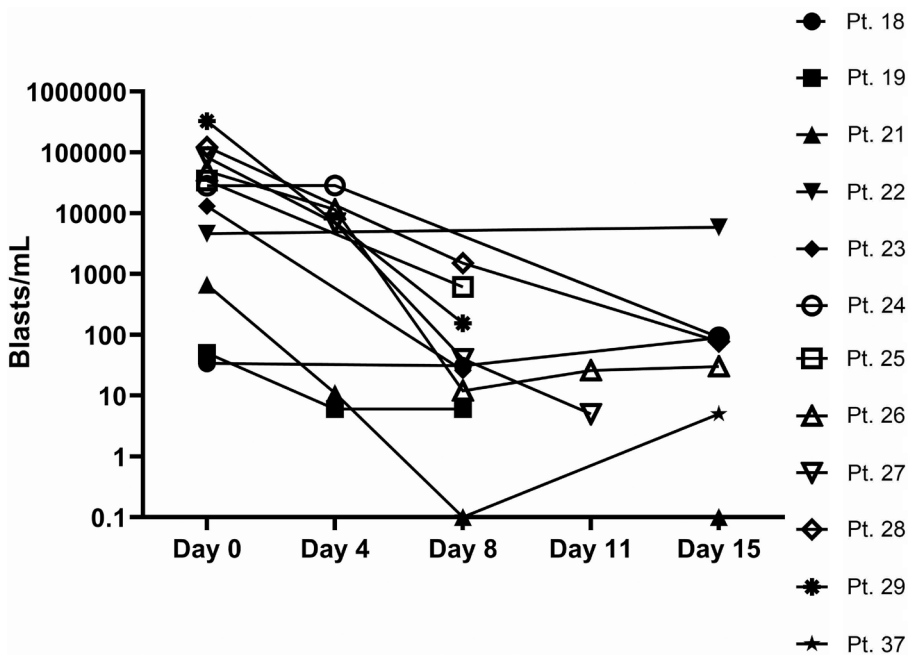

Figure 1. Clearance of leukemic blasts in cerebrospinal fluid at relapse during therapy. The plot shows the number of blasts/mL determined by cerebrospinal fluid (CSF) flow cytometry (FCM) for positive patients (Pt.) with available FCM data (12 of 24) from the first 15 days of therapy after relapse. Each line designates 1 patient. To include the time point, where all blasts were cleared, data points with the value zero were shown as 0.1 on the plot. The data point at day 15 not connected with a line to a day 0 sample, designates Pt. 37 with missing data on CSF sample volume on day 0.

prognostic value of low-level CNS involvement detected by FCM at relapse. Larger prospective studies powered to assess the prognostic effect of CSF FCM positivity at initial relapse and during treatment are therefore needed. Follow-up samples should be collected for all FCM-positive patients, and the slow clearance of blasts in this study show that sampling should be continued beyond day 15 of treatment. To maximize sensitivity of CSF FCM, a minimum of 1-2 mL CSF should be collected directly into TransFix tubes, and FCM should be performed by national MRD laboratories to reduce time from sampling to analysis (optimally less than 48 hours). Especially the ability to monitor blasts clearance in the CSF by FCM during treatment could be a promising tool for guiding the intensity of CNS-directed therapy at relapse, enabling a reduction in neurotoxic treatment in low-risk patients and intensified treatment in high-risk patients.

Authors

Maria Thastrup,[1] Hanne Vibeke Marquart,[2,3] Mette Levinsen,[1] Aina Ulvmoen,[4] Birgitte Klug Albertsen,[5,6] Cornelis Jan Pronk,[7] Marianne Olsen,[8] Peder Wehner,[9] Mervi Taskinen,[10] Bodil Als-Nielsen[1] and Kjeld Schmiegelow[1,3] on behalf of the Nordic Society of Pediatric Hematology and Oncology (NOPHO)

[1]Department of Pediatrics and Adolescent Medicine, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [2]Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [3]Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [4]Department of Pediatrics, Oslo University Hospital, Oslo, Norway; [5]Department of Pediatrics and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark; [6]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; [7]Sweden Childhood Cancer Center, Skaane University Hospital, Lund, Sweden; [8]Pediatric Department, Aalborg University Hospital, Aalborg, Denmark; [9]Department of Pediatric Hematology and Oncology, Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark and [10]Division of Pediatric Hematology, Oncology and Stem Cell Transplantation, Children and Adolescents, Helsinki University Hospital, Helsinki, Finland

Correspondence:
K. SCHMIEGELOW - kjeld.schmiegelow@regionh.dk

https://doi.org/10.3324/haematol.2025.287392

Received: January 20, 2025.
Accepted: July 3, 2025.
Early view: July 10, 2025.

Disclosures

No conflicts of interest to disclose.

Contributions

KS, HVM, and ML designed the study. All authors contributed to sample collection. MT and ML coordinated the study. MT and HVM analyzed the data. MT, HVM and KS interpreted the data and wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the final draft for submission.

Acknowledgments

We thank all Nordic pediatric oncology centers for providing clinical data and CSF samples for flow cytometric analysis. We are grateful to the staff at the Laboratory of Pediatric Oncology and the Department of Immunology, Rigshospitalet, for their help with sample analysis and data registration.

Funding

The study was funded by the Danish Childhood Cancer Foundation, Nordic Cancer Union, Rigshospitalet, Swedish Childhood Cancer Foundation, Danish Cancer Research Foundation, Cancer Research Foundation of Elna and Jørgen Fagerholt Pedersen, Axel Muusfeldt's Foundation, Anders Hasselbalch's Fight Leukemia Foundation, Otto Christensen's Foundation, Memorial Foundation of Merchant M. Brogaard and Wife, Research Foundation of Hasselbalch and Lykkegaard Andersen, Memorial Foundation of Eva and Henry Frænkel, Dagmar Marshall's Foundation, King Christian the Tenth Foundation, and Memorial Trust Foundation of Thora and Viggo Grove. This work is part of the Danish nation-wide research program Childhood Oncology Network Targeting Research, Organization & Life Expectancy (CONTROL) and supported by the Danish Cancer Society (R-257-A14720) and the Danish Childhood Cancer Foundation (2019-5934 and 2020-5769).

Data-sharing statement

Original data can be made available upon request by contacting the corresponding author.

References

1. Winick N, Devidas M, Chen S, et al. Impact of initial CSF findings on outcome among patients with National Cancer Institute standard- and high-risk B-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. J Clin Oncol. 2017;35(22):2527-2534.
2. Otto F, Harrer C, Pilz G, Wipfler P, Harrer A. Role and relevance of cerebrospinal fluid cells in diagnostics and research: state-of-the-art and underutilized opportunities. diagnostics (Basel). 2021;12(1):79.
3. Thastrup M, Duguid A, Mirian C, Schmiegelow K, Halsey C. Central nervous system involvement in childhood acute lymphoblastic leukemia: challenges and solutions.

Leukemia. 2022;36(12):2751-2768.
4. Vora A, Andreano A, Pui CH, et al. Influence of cranial radiotherapy on outcome in children with acute lymphoblastic leukemia treated with contemporary therapy. J Clin Oncol. 2016;34(9):919-926.
5. Krull KR, Hardy KK, Kahalley LS, Schuitema I, Kesler SR. Neurocognitive outcomes and interventions in long-term survivors of childhood cancer. J Clin Oncol. 2018;36(21):2181-2189.
6. Follin C, Erfurth EM. Long-term effect of cranial radiotherapy on pituitary-hypothalamus area in childhood acute lymphoblastic leukemia survivors. Curr Treat Options Oncol. 2016;17(9):50.
7. Thastrup M, Marquart HV, Schmiegelow K. Flow cytometric detection of malignant blasts in cerebrospinal fluid: a biomarker of central nervous system involvement in childhood acute lymphoblastic leukemia. Biomolecules. 2022;12(6):813.
8. Xue L, Shang Q, Lu A, et al. Diagnostic value and prognosis significance of cerebrospinal fluid examination by flow cytometry in pediatric acute lymphoblastic leukemia. Technol Cancer Res Treat. 2023;22:15330338231181025.
9. Ranta S, Nilsson F, Harila-Saari A, et al. Detection of central nervous system involvement in childhood acute lymphoblastic leukemia by cytomorphology and flow cytometry of the cerebrospinal fluid. Pediatr Blood Cancer. 2015;62(6):951-956.
10. Thastrup M, Marquart HV, Levinsen M, et al. Flow cytometric detection of leukemic blasts in cerebrospinal fluid predicts risk of relapse in childhood acute lymphoblastic leukemia: a Nordic Society of Pediatric Hematology and Oncology study. Leukemia. 2020;34(2):336-346.
11. de Haas V, Pieters R, van der Sluijs-Gelling AJ, et al. Flowcytometric evaluation of cerebrospinal fluid in childhood ALL identifies CNS involvement better then conventional cytomorphology. Leukemia. 2021;35(6):1773-1776.
12. Martínez-Laperche C, Gómez-García AM, Lassaletta Á, et al. Detection of occult cerebrospinal fluid involvement during maintenance therapy identifies a group of children with acute lymphoblastic leukemia at high risk for relapse. Am J Hematol. 2013;88(5):359-364.
13. Gabelli M, Disarò S, Scarparo P, et al. Cerebrospinal fluid analysis by 8-color flow cytometry in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2019;60(11):2825-2828.
14. Cancela CSP, Murao M, Assumpção JG, et al. Immunophenotyping of the cerebrospinal fluid as a prognostic factor at diagnosis of acute lymphoblastic leukemia in children and adolescents. Pediatr Hematol Oncol. 2017;34(2):53-65.