Pediatric Blood & Cancer

ONCOLOGY: BRIEF REPORT

Does minimal central nervous system involvement in childhood acute lymphoblastic leukemia increase the risk for central nervous system toxicity?

Stavroula Anastasopoulou[1,2] | Arja Harila-Saari[3] | Bodil Als-Nielsen[4] | Mats Anders Eriksson[1,5] | Mats Heyman[1,2] | Inga Maria Johannsdottir[6] | Hanne Vibeke Marquart[7] | Riitta Niinimäki[8] | Cornelis Jan Pronk[9] | Kjeld Schmiegelow[4,10] | Goda Vaitkeviciene[11] | Maria Thastrup[4] | Susanna Ranta[1,2]

[1]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden

[2]Department of Women's and Children's Health, Childhood Cancer Research Unit, Karolinska Institutet, Stockholm, Sweden

[3]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

[4]Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark

[5]Department of Women's and Children's Health, Neuropediatric Unit, Karolinska Institutet, Stockholm, Sweden

[6]Department of Pediatric Hematology/Oncology, Oslo University Hospital, Oslo, Norway

[7]Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

[8]Department of Children and Adolescents, Oulu University Hospital and University of Oulu, PEDEGO Research Unit, Oulu, Finland

[9]Childhood Cancer Centre, Skåne University Hospital, Lund, Sweden

[10]Institute of Clinical Medicine, Faculty of Medicine, University of Copenhagen, Copenhagen, Denmark

[11]Children's Hospital, Affiliate of Vilnius University Hospital Santaros Klinikos and Vilnius University, Vilnius, Lithuania

Correspondence
Stavroula Anastasopoulou, Department of Women's and Children's Health, Childhood Cancer Research Unit, Karolinska Institute, Neuropediatric Unit, QB82 Karolinska vägen 37A, 171 76 Stockholm, Sweden.
Email: stavroula.anastasopoulou@ki.se

Authors Maria Thastrup and Susanna Ranta contributed equally to this study.

Abstract

Central nervous system (CNS) involvement in childhood acute lymphoblastic leukemia (ALL) implicates enhanced intrathecal chemotherapy, which is related to CNS toxicity. Whether CNS involvement alone contributes to CNS toxicity remains unclear. We studied the occurrence of all CNS toxicities, seizures, and posterior reversible encephalopathy syndrome (PRES) in children with ALL without enhanced intrathecal chemotherapy with CNS involvement ($n = 64$) or without CNS involvement ($n = 256$) by flow cytometry. CNS involvement increased the risk for all CNS toxicities, seizures, and PRES in univariate analysis and, after adjusting for induction therapy, for seizures

(hazard ratio [HR] = 3.33; 95% confidence interval [CI]: 1.26–8.82; $p = 0.016$) and PRES (HR = 4.85; 95% CI: 1.71–13.75; $p = 0.003$).

KEYWORDS
CNS leukemia, CNS toxicity, flow cytometric immunophenotyping, pediatric acute lymphoblastic leukemia

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) can involve extramedullary sites including the central nervous system (CNS).[1] Traditionally, the diagnosis of leukemic cells in the cerebrospinal fluid (CSF) is performed by cytomorphology (CM). Flow cytometric immunophenotyping (FCI) is more sensitive than CM and can detect low levels of blasts in CSF despite normal CM findings but is not routinely used in clinical diagnostics.[2–6]

Current treatment protocols include CNS-directed chemotherapy for all patients to reduce relapse risk, even in the absence of signs or symptoms of CNS leukemia, while those with known CNS involvement receive enhanced intrathecal chemotherapy.[1,3] Recent studies suggest that CNS leukemia defined by CM increases the risk for CNS toxicities and early posterior reversible encephalopathy syndrome (PRES).[7,8] Whether this is due to leukemic cells in the CSF per se or the enhanced CNS-directed treatment remains unclear.[9]

We explored here if leukemic involvement of the CSF with the more sensitive method, FCI, is associated with CNS toxicity and if minimal leukemic involvement in the CSF by FCI alone, without enhanced intrathecal chemotherapy, increases the risk for CNS toxicities. We hypothesized that the presence of blasts in the CSF in patients with CNS by FCI increases the risk for CNS toxicities during induction.

2 | METHODS

Children aged between 1 and 17.9 years, diagnosed with ALL between 2008 and 2015 and treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL2008 protocol, with data from diagnostic lumbar puncture by both CSF FCI and CM, were included. Patients with CNS toxicities were identified through a prospective online registration system.[1,7,10] CNS toxicities were grouped into three categories: "all CNS toxicities" including patients with any CNS toxicity, "seizures" including patients with isolated seizures and those with seizures secondary to other CNS toxicity, and "PRES."

CNS involvement at diagnosis as reported in the NOPHO registry is determined by the presence of leukemic blasts in the CSF by CM or recently appearing neurological symptoms and/or pathological neuroimaging findings, as well as eye involvement.[1] Patients without CNS involvement were classified as CNS1. Patients with CNS leukemia were classified as CNS2 if they had <5/μl cells in the CSF and leukemic blasts identified by CM and as CNS3 if they had ≥5/μl cells in the CSF and leukemic blasts by CM, or clinical/ radiological signs of leukemic CNS involvement. Patients classified as CNS2 or CNS3 received extra intrathecal chemotherapy until the CSF was free of leukemic cells, and CNS3 patients continued enhanced intrathecal therapy throughout the leukemia treatment.[1]

Patients with data on CSF by FCI at diagnosis were identified from a previous study and combined with registry data.[4,7,10] Patients with ≥10 blasts in the CSF by FCI were defined as having CNS involvement (CNS$_{flow+}$) and patients with <10 blasts in the CSF were defined as not having CNS involvement (CNS$_{flow-}$).[4]

Statistical analyses were performed using SPSS, version 26.0. Group differences were assessed by the Mann–Whitney U test and the chi-square test, as appropriate. Time to CNS toxicity was defined as the time from ALL diagnosis until the day of CNS toxicity censoring for death, relapse, stem cell transplantation, secondary malignancy, or end of follow-up, whichever occurred first. The induction period was defined as the first 5 weeks from treatment start. Risk factors for CNS toxicities during the entire follow-up period were evaluated using Cox proportional hazards models. Univariate analyses and adjustments for clinically relevant risk factors were performed. The association of stratification to block-treatment with late CNS toxicities occurring after induction was evaluated by Cox proportional hazards models excluding the cases with early CNS toxicities during induction. The association between CNS leukemia by FCI and the risk of early CNS toxicities during the induction period was evaluated with logistic regression, excluding patients who died during induction. Two-sided p-values < 0.05 were considered statistically significant.

Ethical review committees in all countries have approved the NOPHO registry, ALL2008 protocol, and the FCI study.

3 | RESULTS

The study included 370 children, of whom 320 were classified as CNS1 by CM including 256 (80%) without (CNS1$_{flow-}$) and 64 (20%) with (CNS1$_{flow+}$) blasts in the CSF by FCI. Fifty patients were classified as CNS2 or CNS3 by CM and/or clinical symptoms and neuroimaging, 36 of whom had blasts in the CSF by FCI (discrepancy background as previously described[4]).

Overall, 38 patients (38/370, 10.3%) reported at least one episode of CNS toxicity (22 with seizures, 16 of these with PRES; two additional patients had PRES without seizures). Among CNS1 patients, 33 children (33/320, 10.3%) had CNS toxicity (18 with seizures, 14 of

TABLE 1 Comparison of clinical characteristics in patients with acute lymphoblastic leukemia (ALL) and CNS1 status with and without leukemic cells in the cerebrospinal fluid by flow cytometric immunophenotyping

Patients	ALL	$CNS1_{flow-}$	$CNS1_{flow+}$	p^{*}
Total (n)	320	256	64	
Age median and range (years)	4.0 (1.0–17.0)	4.0 (1.0–17.0)	4.0 (1.0–16.0)	0.647
Sex				
Male (%)	173 (54.1)	145 (56.6)	28 (43.8)	
Female (%)	147 (45.9)	111 (43.4)	36 (56.3)	0.064
WBC				
$<100 \times 10^9$/L (%)	291 (90.9)	244 (95.3)	47 (73.4)	
$>100 \times 10^9$/L (%)	29 (9.1)	12 (4.7)	17 (26.6)	<0.001
Immunophenotype				
BCP (%)	286 (89.4)	238 (93.0)	48 (75.0)	
T cell (%)	34 (10.6)	18 (7.0)	16 (25.0)	<0.001
Induction therapy[**]				
Prednisolone (%)	266 (84.7)	226 (89.7)	40 (64.5)	
Dexamethasone (%)	48 (15.3)	26 (10.3)	22 (35.5)	<0.001
Stratification into block treatment at the end of induction				
Non-block treatment (%)	272 (85.0)	220 (85.9)	52 (81.3)	
Block treatment (%)	48 (15.0)	36 (14.1)	12 (18.8)	0.348

[*]p Calculated by Mann–Whitney U test for age and WBC and by chi-square for sex, immunophenotype, induction therapy, and stratification into block treatment at the end of induction.
[**]Missing values for six patients.
Abbreviations: ALL, acute lymphoblastic leukemia; BCP, B-cell precursor; CNS, central nervous system; CNS1, patients without CNS leukemia by cytomorphology; $CNS1_{flow-}$, patients with CNS1 and negative flow cytometric immunophenotyping; $CNS1_{flow+}$, patients with CNS1 and positive flow cytometric immunophenotyping; WBC, white blood cells.

these with PRES; two additional patients had PRES without seizures) (**Table** S1).

When exploring clinical factors and risks for CNS toxicities, older age and stratification to block treatment after induction were associated with CNS toxicities in patients with CNS1 (**Table** S2). In CNS1 patients, CSF FCI positivity was significantly more common in those classified as high-risk patients at diagnosis (white blood cell count [WBC] $\geq 100 \times 10^9$/L at diagnosis and/or T-cell immunophenotype) and consequently received induction therapy with dexamethasone (Table 1). Since our cohort was too small for simultaneous multiple adjustments, induction therapy was chosen for multivariate analyses as it accounts for both WBC and immunophenotype.

We first studied whether CNS leukemia determined by FCI was associated with CNS toxicities. Having blasts in the CSF by FCI increased the risk for seizures and PRES in univariate analyses and, after adjusting for the type of induction therapy, remained significant for PRES (**Table** S3).

We then proceeded to study if having leukemic blasts in the CSF at diagnosis alone, without enhanced intrathecal treatment (CNS1), increased the risk of CNS toxicity by comparing the occurrence of any CNS toxicity, seizures, or PRES in 64 children who had positive CSF FCI ($CNS1_{flow+}$) with 256 children who had negative CSF FCI ($CNS1_{flow-}$). $CNS1_{flow+}$ was a significant risk factor for CNS toxicities in all three

groups in univariate analyses and for seizures and PRES also after adjusting for induction therapy. $CNS1_{flow+}$ remained a significant risk factor for all three groups after adjusting separately for age (Table 2). Further, $CNS1_{flow+}$ remained a significant risk factor for late PRES after adjusting for stratification to block treatment, but the cohort was too small for confident conclusions (hazard ratio [HR] = 6.21 (95% confidence interval [CI]: 1.75–22.03), $p = 0.005$).

Finally, we tested if $CNS1_{flow+}$ was associated with CNS toxicity during induction compared to those with $CNS1_{flow-}$. Unfortunately, the number of cases with CNS toxicities was too low to draw any firm conclusions (seizures, $n = 5$: odds ratio [OR] = 6.342 (95% CI: 1.035–38.844), $p = 0.046$ and PRES, $n = 6$: OR = 4.086 (95% CI: 0.804–20.769), $p = 0.090$).

4 | DISCUSSION

The role of CNS leukemia in the risk of CNS toxicity is unclear. Some recent studies support that CNS leukemia is associated with an increased risk of acute or early CNS toxicity, but other studies show that CNS leukemia does not implicate CNS toxicity.[7,8,11] This might reflect differences in protocols including intrathecal administration of methotrexate and cytarabine.[9]

TABLE 2 Risk of central nervous system (CNS) toxicity in cases with CNS1 and leukemic cells in the cerebrospinal fluid by flow cytometric immunophenotyping

	Controls (n)	Cases (n)	Univariate HR (95% CI; p)	Multivariate HR (95% CI; p)*	Multivariate HR (95% CI; p)**
CNS1$_{flow+}$ vs. CNS1$_{flow-}$					
All CNS toxicities	287	33			
CNS1$_{flow-}$, n (%)	234 (81.5)	22 (66.7)	Ref.	Ref.	Ref.
CNS1$_{flow+}$, n (%)	53 (18.5)	11 (33.3)	2.08 (1.01–4.28; 0.048)	2.35 (1.13–4.87; 0.022)	1.90 (0.88–4.10; 0.101)
Seizures	287	18			
CNS1$_{flow-}$, n (%)	234 (81.5)	10 (55.6)	Ref.	Ref.	Ref.
CNS1$_{flow+}$, n (%)	53 (18.5)	8 (44.4)	3.34 (1.32–8.47; 0.011)	3.83 (1.50–9.76; 0.005)	3.33 (1.26–8.82; 0.016)
PRES	287	16			
CNS1$_{flow-}$, n (%)	234 (81.5)	7 (43.8)	Ref.		Ref.
CNS1$_{flow+}$, n (%)	53 (18.5)	9 (56.3)	5.30 (1.98–14.24; < 0.001)	6.13 (2.26–16.64; < 0.001)	4.85 (1.71–13.75; 0.003)

*Adjusted for age.
**Adjusted for induction therapy. High-risk induction with dexamethasone included 3 weeks dexamethasone 10 mg/m^2 as opposed to non-high-risk induction with 4 weeks of prednisolone 60 mg/m^2, otherwise there were no differences in systemic treatment. Abbreviations: CI, confidence interval; CNS , central nervous system; CNS1, patients without CNS leukemia by cytomorphology; CNS1$_{flow-}$, patients with CNS1 and negative flow cytometric immunophenotyping; CNS1$_{flow+}$, patients with CNS1 and positive flow cytometric immunophenotyping; HR, hazard ratio; PRES, posterior reversible encephalopathy syndrome.

Minimal leukemic CNS involvement by FCI has been shown to increase the risk of leukemia relapse.[4] The present finding shows that minimal CNS leukemia per se is additionally associated with CNS toxicity. CNS leukemia is related to higher methotrexate concentrations in the CSF and consequently higher risk for CNS toxicities.[12] Further studies on methotrexate clearance in CSF could elucidate whether minimal CNS leukemia also increases methotrexate concentrations.

Leukemic cells produce significant amounts of vascular endothelial growth factor (VEGF), which increases the permeability of cerebral vessels, including disruption of the blood–brain barrier, by acting directly on endothelial cells.[13,14] Increased cerebrovascular permeability is believed to be the main underlying mechanism of PRES.[15] The higher levels of VEGF detected in the CSF of patients with CNS involvement could contribute to the higher risk for PRES also in patients with minimal leukemic involvement.[13,16]

The main limitation of this study is the small cohort, disallowing adjusting for several variables in multivariate analyses. Strengths include the well-defined study cohort treated according to the same protocol and detailed data collected on CNS toxicities.

5 | CONCLUSION

Our study suggests that minimal CNS leukemia without enhanced CNS-directed chemotherapy increases the risk for CNS toxicity, especially PRES. The increased risks of leukemia relapse and adverse events in patients with minimal CNS leukemia motivate future studies to better understand the underlying mechanisms and determine how CNS-directed chemotherapy should be tailored to these patients.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Stavroula Anastasopoulou https://orcid.org/0000-0002-4210-0064
Arja Harila-Saari https://orcid.org/0000-0003-2767-5828
Riitta Niinimäki https://orcid.org/0000-0003-0190-5664
Kjeld Schmiegelow https://orcid.org/0000-0002-0829-4993
Susanna Ranta https://orcid.org/0000-0001-7854-0371

REFERENCES

1. Toft N, Birgens H, Abrahamsson J, et al. Risk group assignment differs for children and adults 1–45 yr with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol. *Eur J Haematol.* 2013;90(5):404–412.
2. Lenk L, Alsadeq A, Schewe DM. Involvement of the central nervous system in acute lymphoblastic leukemia: opinions on molecular mechanisms and clinical implications based on recent data. *Cancer Metastasis Rev.* 2020;39(1):173–187.
3. 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.

4. 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.

5. Thastrup M, Marquart HV, Levinsen M, et al. Flow cytometric analysis of cerebrospinal fluid improves detection of leukaemic blasts in infants with acute lymphoblastic leukaemia. *Br J Haematol*. 2021;195:119–122.

6. Levinsen M, Taskinen M, Abrahamsson J, et al. Clinical features and early treatment response of central nervous system involvement in childhood acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2014;61(8):1416–1421.

7. Anastasopoulou S, Eriksson MA, Heyman M, et al. Posterior reversible encephalopathy syndrome in children with acute lymphoblastic leukemia: clinical characteristics, risk factors, course, and outcome of disease. *Pediatr Blood Cancer*. 2018;66:e27594.

8. Banerjee J, Niinimaki R, Lahteenmaki P, et al. The spectrum of acute central nervous system symptoms during the treatment of childhood acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. 2020;67(2):e27999.

9. Peddi PF, Peddi S, Santos ES, Morgensztern D. Central nervous system toxicities of chemotherapeutic agents. *Expert Rev Anticancer Ther*. 2014;14(7):857–863.

10. Anastasopoulou S, Heyman M, Eriksson MA, et al. Seizures during treatment of childhood acute lymphoblastic leukemia: a population-based cohort study. *Eur J Paediatr Neurol*. 2020;27:72–77.

11. Mateos MK, Marshall GM, Barbaro PM, et al. Methotrexate-related central neurotoxicity: clinical characteristics, risk factors and genome-wide association study in children treated for acute lymphoblastic leukemia. *Haematologica*. 2021;107:635–643.

12. Bleyer WA, Drake JC, Chabner BA. Neurotoxicity and elevated cerebrospinal-fluid methotrexate concentration in meningeal leukemia. *N Engl J Med*. 1973;289(15):770–773.

13. Deak D, Gorcea-Andronic N, Sas V, et al. A narrative review of central nervous system involvement in acute leukemias. *Ann Transl Med*. 2021;9(1):68.

14. Feng S, Huang Y, Chen Z. Does VEGF secreted by leukemic cells increase the permeability of blood-brain barrier by disrupting tight-junction proteins in central nervous system leukemia?. *Med Hypotheses*. 2011;76(5):618–621.

15. Fugate JE, Rabinstein AA. Posterior reversible encephalopathy syndrome: clinical and radiological manifestations, pathophysiology, and outstanding questions. *Lancet Neurol*. 2015;14(9):914–925.

16. Tang YT, Jiang F, Guo L, Si MY, Jiao XY. Expression and significance of vascular endothelial growth factor A and C in leukemia central nervous system metastasis. *Leuk Res*. 2013;37(4):359–366.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.