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

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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.
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 Pediatric 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 diagnostic lumbar puncture by both CSF FCI and CM, with normal CM findings but is not routinely used in clinical diagnostics.\(^2\)\^-\(^6\)

The cases with early CNS toxicities during induction was evaluated by 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 (CNS\(_{1\text{low}}\)) and 64 (20%) with (CNS\(_{1\text{flow}}\)) 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\(^1\)).

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

(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
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] ≥100 x 10⁹/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 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

<table>
<thead>
<tr>
<th>Patients</th>
<th>ALL</th>
<th>CNS1_flow-</th>
<th>CNS1_flow+</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>320</td>
<td>256</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Age median and range (years)</td>
<td>4.0 (1.0–17.0)</td>
<td>4.0 (1.0–17.0)</td>
<td>4.0 (1.0–16.0)</td>
<td>0.647</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>173 (54.1)</td>
<td>145 (56.6)</td>
<td>28 (43.8)</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>147 (45.9)</td>
<td>111 (43.4)</td>
<td>36 (56.3)</td>
<td></td>
</tr>
<tr>
<td>WBC &lt; 100 x 10⁹/L (%)</td>
<td>291 (90.9)</td>
<td>244 (95.3)</td>
<td>47 (73.4)</td>
<td></td>
</tr>
<tr>
<td>&gt; 100 x 10⁹/L (%)</td>
<td>29 (9.1)</td>
<td>12 (4.7)</td>
<td>17 (26.6)</td>
<td></td>
</tr>
<tr>
<td>Immunophenotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BCP (%)</td>
<td>286 (89.4)</td>
<td>238 (93.0)</td>
<td>48 (75.0)</td>
<td></td>
</tr>
<tr>
<td>T cell (%)</td>
<td>34 (10.6)</td>
<td>18 (7.0)</td>
<td>16 (25.0)</td>
<td></td>
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<tr>
<td>Induction therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone (%)</td>
<td>266 (84.7)</td>
<td>226 (89.7)</td>
<td>40 (64.5)</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone (%)</td>
<td>48 (15.3)</td>
<td>26 (10.3)</td>
<td>22 (35.5)</td>
<td></td>
</tr>
<tr>
<td>Stratification into block treatment at the end of induction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-block treatment (%)</td>
<td>272 (85.0)</td>
<td>220 (85.9)</td>
<td>52 (81.3)</td>
<td></td>
</tr>
<tr>
<td>Block treatment (%)</td>
<td>48 (15.0)</td>
<td>36 (14.1)</td>
<td>12 (18.8)</td>
<td></td>
</tr>
</tbody>
</table>

¹p 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.

²**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.
The present finding shows 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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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