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Prognostic significance of the initial cerebro-spinal fluid (CSF) involvement of children with acute lymphoblastic leukaemia (ALL) treated without cranial irradiation: Results of European Organization for Research and Treatment of Cancer (EORTC) Children Leukemia Group study 58881

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ABSTRACT

Aim of the study: To evaluate the prognostic significance of the initial cerebro-spinal fluid (CSF) involvement of children with ALL enrolled from 1989 to 1996 in the EORTC 58881 trial.
Patients and methods: Patients (2025) were categorised according to initial central nervous system (CNS) status: CNS-1 (CNS negative, $n = 1866$), CNS-2 (<5 leucocytes/mm^3, CSF with blasts, $n = 50$), CNS-3 (CNS positive, $n = 49$), TLP+ (TLP with blasts, $n = 60$). CNS-directed therapy consisted in intravenous (i.v.) methotrexate (5 g/sqm) in 4–10 courses, and intrathecal methotrexate injections (10–20), according to CNS status. Cranial irradiation was omitted in all patients.
Results: In the CNS1, TLP+, CNS2 and CNS3 group the 8-year EFS rate (SE%) was 69.7% (1.1%), 68.8% (6.2%), 71.3% (6.5%) and 68.3% (6.2%), respectively. The 8-year incidence of isolated CNS relapse (SE%) was 3.4% (0.4%), 1.7% (1.7%), 6.1% (3.5%) and 9.4% (4.5%), respectively, whereas the 8-year isolated or combined CNS relapse incidence was 7.6% (0.6%),

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3.5% (2.4%), 10.2% (4.4%) and 11.7% (5.0%), respectively. Patients with CSF blasts had a higher rate of initial bad risk features. Multivariate analysis indicated that presence of blasts in the CSF had no prognostic value: (i) for EFS and OS; (ii) for isolated and isolated or combined CNS relapse; WBC count < 25×10^9/L and Medac E-coli asparaginase treatment were each related to a lower CNS relapse risk.

Conclusions: The presence of initial CNS involvement has no prognostic significance in EORTC 58881. Intensification of CNS-directed chemotherapy, without CNS radiation, is an effective treatment of initial meningeal leukaemic involvement.

1. Introduction

Presymptomatic treatment of leukaemia in the central nervous system (CNS) remains a challenge in ALL of childhood.[1] Cranial irradiation (XRT) in combination with intrathecal (IT) chemotherapy has long been the approach adopted by most groups in Europe and North America and has markedly reduced the incidence of meningeal relapse.[2,3] Recently, the use of XRT has become contentious because of its late adverse effects, especially in young children: neurocognitive dysfunction, growth impairment, precocious puberty and enhanced risk of developing brain tumours.[4–6] These late complications have led to the gradual reduction of XRT dose or even to its omission in patients with standard risk of relapse.[5,7,8] However, in most current clinical trials, cranial XRT is still recommended for patients at high risk of relapse within the CNS, especially those with leukaemic involvement of the CNS at the time of diagnosis (3–5% of the cases).

Among the initial factors which predict a higher risk of CNS relapse, the presence of leukaemic cells in a CSF containing less than 5 leucocytes/mm^3 remains controversial. It was associated with an enhanced risk of CNS relapse in some but not all clinical studies. Likewise, a poorer event-free survival (EFS) was associated with the presence of blasts in traumatic lumbar punctuates at diagnosis of ALL.[9]

In EORTC CLG, after we had shown that cranial XRT failed to provide any benefit to medium- and high-risk patients having received high-dose methotrexate (HD MTX), we have deleted that therapy component from the treatment regimen of all ALL patients, including those with overt CNS leukaemia involvement. EORTC 58881 was the first trial in which this strategy was implemented.[10]

The aim of this retrospective study is to determine the prognostic significance of blasts with any number of leucocytes, with or without erythrocytes, in the CSF of patients recruited in EORTC CLG 58881. The time between the study closure and this analysis allowed us to identify possible late relapses, which is particularly important in a strategy which omits radiotherapy.

2. Patients and methods

2.1. Patients

From January 1989 to November 1998, 2070 consecutive children (1165 boys, sex ratio, 1.29) less than 18 years of age with newly diagnosed ALL from 28 paediatric departments (France, Belgium, and Portugal) were prospectively enrolled in the multicentre trial EORTC 58881. Bone marrow smears, immunophenotypes and cytogenetics were reviewed centrally. Identification of leukaemic blasts in the CSF was done by conventional cytology. The patients were stratified into low- and increased-risk categories according to their immunophenotype and their risk-factor was calculated as a function of blood blast count, hepatomegaly and splenomegaly as proposed by Langermann and colleagues[10,11]. A very high-risk group (VHR) was defined by the presence of at least one of the following criteria: more than 1×10^9/L blasts in the peripheral blood after 7 d of treatment with prednisolone and one intrathecal injection of methotrexate on day 1 (called 'poor responder to the prephase'), presence of a t(4;11) or a t(9;22) translocation or near-haploidy in the leukaemic clone or the failure to achieve complete remission (CR) at completion of induction therapy.

2.2. CNS status

All patients underwent diagnostic lumbar puncture and intrathecal methotrexate injection on the first day of induction therapy. According to CNS status, patients were classified into the following groups: CNS-1 (<5 white blood cells [WBCs]/mm^3 with no leukaemic blast cells after cytocentrifugation); CNS-2 (lumbar puncture not traumatic (i.e. <100 RBCs/mm^3); <5 WBCs/mm^3 with presence of leukaemic blasts after cytocentrifugation); CNS-3 (lumbar puncture not traumatic (i.e. <100 RBCs/mm^3); $\geqslant$5 WBCs/mm^3 with presence of leukaemic blasts after cytocentrifugation); TLP+ (traumatic lumbar puncture [$\geqslant$100 RBCs/mm^3] with presence of leukaemic blasts after cytocentrifugation). TLP-patients (traumatic lumbar puncture [$\geqslant$100 RBCs/mm^3] without the presence of leukaemic blasts after cytocentrifugation) were included in the CNS-1 group. The CNS status of patients for whom 2 consecutive traumatic lumbar punctures contained leukaemic blasts was classified as TLP++. Patients with cranial nerve palsies attributed to leukaemic involvement were considered as CNS-3 status, irrespective of the presence of blasts in the CSF.

2.3. Treatment programmes

The treatment regimen, adapted from the Berlin–Frankfurt–Munster (BFM)-protocol, and the design of the clinical trial have been described in detail recently.[10] An overview of the successive courses of chemotherapy for low- and increased-risk patients is provided in Table 1. The treatment

Table 1 – EORTC-CLG 58881: treatment protocols for low- and increased-risk patients.

Drug	Dose	Days of administration
Induction: protocol I A		
Prednisolone (PO)	60 mg/m^2	1–28
Vincristine (IV)	1.5 mg/m^2	8, 15, 22, 29 (max. 2 mg)
Daunorubicine	30 mg/m^2	8, 15, 22, 29
Methotrexate (intrathecal)	12 mg[a]	1, 8, 22
According to randomisation		
E-coli-asparaginase (IV)	10,000 IU/m^2	12, 15, 18, 22, 25, 29, 32, 35
or		
Erwinia-asparaginase (IV)	10,000 IU/m^2	12, 15, 18, 22, 25, 29, 32, 35
Consolidation: protocol IB		
Cyclophosphamide (IV)	1000 mg/m^2	36–63
Cytarabine (IV)	75 mg/m^2	38–41, 45–48, 52–55, 59–62
6-Mercaptopurine (PO)	60 mg/m^2	36–63
Methotrexate (intrathecal)	12 mg[a]	38, 52
Interval therapy		
6-Mercaptopurine (PO)	25 mg/m^2	1–56
Methotrexate (24 h IV infusion with leucovorin rescue)	5000 mg/m^2	8, 22, 36, 50
Methotrexate (intrathecal)	12 mg[a]	9, 23, 37, 51
According to randomisation for increased-risk patients		
Cytarabine (IV) or no cytarabine	2000 mg/m^2	9, 10, 23, 24, 37, 38, 51, 52
Reinduction:protocol II		
Dexamethasone (PO)	10 mg/m^2	1–21
Vincristine (IV)	1.5 mg/m^2 (max. 2 mg)	8, 15, 22, 29
Doxorubicin (IV)	30 mg/m^2	8, 15, 22, 29
Methotrexate (intrathecal)	12 mg[a]	38
Cyclophosphamide (IV)	1000 mg/m^2	36
Cytarabine (IV)	75 mg/m^2	38–41, 45–48,
6-Thioguanine (PO)	60 mg/m^2	36–49
According to randomisation		
E-coli-asparaginase (IV)	10,000 IU/m^2	8, 11, 15, 18
or		
Erwinia-asparaginase (IV)	10,000 IU/m^2	8, 11, 15, 18

Maintenance therapy was a combination of daily oral mercaptopurine adjusted to maintain leucocytes between 2 and 3×10^9/L and methotrexate 20 mg/m^2 once a week. According to randomisation, some patients received intravenous mercaptopurine 1 g/m^2 every 4 weeks.

[a] Doses were adjusted for children under 3 years of age.

of VHR patients called for eight rotating consolidation courses followed by maintenance chemotherapy or, when a HLA compatible sibling donor was available, for stem-cell transplantation after the second consolidation course.

Courses of HD MTX (5 g/m^2 over 24 h) were given to all patients: 4 times after the first consolidation course for low- and increased-risk patients and 10 times for the VHR patients. The total number of IT injections was 10 (methotrexate) for low- and increased-risk patients and 20 (methotrexate: 14; triple IT therapy: methotrexate, aracytine, and prednisone: 6) for the VHR patients. CNS status other than CNS-1 did not call for any upgrading in the risk-adapted subgroup stratification. However, CNS-3 patients received additional CNS-directed therapy: IT injections (MTX) every 4th day during prephase and induction if necessary until disappearance of leukaemic blasts from the CSF, two additional IT injections during induction and two during consolidation and 5 courses of HD MTX during maintenance therapy. CNS-2 and TLP+ patients were treated as CNS-3 patients if leukaemic blasts were still present in the CSF at the first control lumbar puncture performed 3 d after the initial IT methotrexate injection. No XRT was used, neither to the central nervous system (CNS) nor to the testes.

In addition, the trial called for three successive randomisations: (1) part of the patients were randomised at the start of therapy to receive either Erwiniase or Medac E-coli-asparaginase; (2) increased risk patients were randomised to the addition or not of Ara-C to the HD MTX courses during interval therapy and (3) all non-VHR patients were randomised to the addition or not of monthly intravenous courses of 6-mercaptopurine during maintenance therapy.[12–14] Increased-risk patients with initial CNS-3 status, but not those with initial CNS-2 or TLP+ status, were not randomised for the second question but were all treated with the addition of HD-Ara-C to the HD MTX courses during interval therapy. For all children remaining in the first complete remission, treatment was stopped at 2 years from its initiation. To ensure comparability with other studies, the results were also analysed according to the National Cancer Institute (NCI) risk groups for ALL.[1] Informed consent by the parents or the legal

guardian was required for registration in the trial. The protocol was approved by the respective institutional review boards and ethical committees.

2.4. Statistical analysis

Event-free survival (EFS) was calculated from the date of complete remission (CR) until the first event (relapse, death of any cause), or until the last follow-up (censored observation). For patients who failed to reach CR by the end of protocol I, the failure was considered as an event at time 0. Overall survival (OS) was defined as the time from registration in the study to the date of death or the last follow-up examination (censored observation). Kaplan–Meier technique was used to estimate survival-type distributions (EFS and OS) and the standard errors (SE) of the estimates were obtained via the Greenwood formula.[15] The differences between Kaplan–Meier curves were tested for statistical significance by the two-tailed log-rank test.[15] The estimates of the incidence of isolated CNS relapse and of isolated or combined CNS relapse were obtained using the competing risk methods, and their comparison was done using the Gray test.[15] The Cox Proportional Hazards model was used to obtain the estimate and the 95% confidence interval (CI) of the hazard ratio (HR) of the instantaneous event rate in one group versus that in another group, as specified by a given variable.[15] In Cox multivariate analysis, the following variables were considered: initial WBC (<25, 25–99, $\geqslant 100 \times 10^9$/L), immunophenotyping (T versus B-lineage ALL), NCI risk group, initial VHR features (presence versus absence), type of asparaginase and CNS-status. All analyses were based on the intent-to-treat principle. The SAS 9.1 statistical software was used.

3. Results

Median duration of the follow-up was 7.5 years. Of the 2070 patients included in this study, 2025 were evaluable for initial CNS status and stratified in low-risk (678 patients, 33%), increased-risk (1032 patients, 51%) and VHR (315 patients, 15%) subgroups (Table 2). There were 1866 (92.1%) CNS-1, 50 (2.4%) CNS-2, 49 (2.4%) CNS-3 and 60 (2.9%) TLP+ patients. Those with CNS-2, CNS-3 or TLP+ taken altogether had more unfavourable features than CNS-1 patients: WBC counts above 100×10^9/L (36% versus 12%), NCI high risk (60% versus 34%), VHR features (32% versus 14%), T-lineage (28% versus 13%). Twenty-one (35%) of the TLP+ patients and 17 (34%) of the CNS-2 patients were treated as CNS-3 patients because of the persistence of at least one leukaemic blast in the spinal tap performed 3 d after the initial one.

The overall 8-year EFS rate was 69.6% (SE 1%). The 8-year EFS rates (SE%) for patients in each CNS group were as follows: 69.7% (1.1%) in CNS-1, 68.8% (6.2%) in TLP+, 71.3% (6.5%) in CNS-2, and 68.3% (6.2%) in CNS-3 (Fig. 1). The 8-year EFS rate of patients with blasts at diagnosis in the CSF (TLP+, CNS-2 and CNS-3) was 69.2% (3.7%), and was not significantly different ($p = 0.45$) from that of those with CNS-1 status (Fig. 2).

The overall 8-year OS rate (SE%) was 80.4% (0.9%). The 8-year OS rates (SE%) for patients in each CNS group were as follows: 80.9% (0.9%) in CNS-1, 79.5% (5.3%) in TLP+, 77.0% (6.1%) in CNS-2 and 67.4% (6.8%) in CNS-3. The 8-year OS rate of patients with blasts in the CSF at diagnosis (TLP+, CNS-2 and CNS-3) was 74.8% (3.6%), and was lower (logrank test: $p = 0.047$) than that of those with CNS-1 status.

Table 2 – Patients characteristics according to CNS status.

Characteristics	All		CNS1		TLP+		CNS2		CNS3	
	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
All	2025		1866		60		50		49	
Sex										
Male	1142	56	1050	56	35	58	29	58	24	49
Female	891	44	816	44	25	42	21	42	25	51
Age (years)										
<1	55	3	33	2	10	17	6	12	6	12
1–10	1619	80	1513	81	38	63	39	78	29	59
>10	351	17	320	17	12	20	5	10	14	29
Immunology										
B-lineage	1734	86	1621	87	44	73	40	80	29	59
T-lineage	291	14	245	13	16	27	10	20	20	41
WBC ($\times 10^9$/L)										
<100	1746	86	1644	88	39	65	38	76	25	51
$\geqslant$100	279	24	222	12	21	35	12	24	24	49
Risk group										
Low-risk	678	33	656	35	6	10	9	18	7	14
Increased-risk	1032	52	946	51	31	52	30	60	25	51
VHR	315	15	264	14	23	38	11	22	17	35
NCI features										
Standard-risk	1292	63	1228	66	23	38	32	64	9	18
High-risk	733	37	638	34	37	62	18	36	40	82

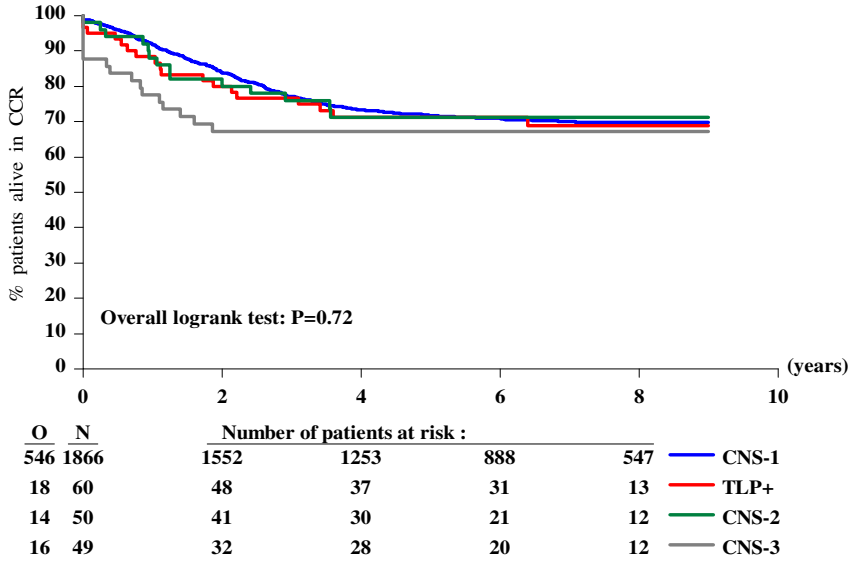

Fig. 1 – Event-free survival according to initial CNS status.

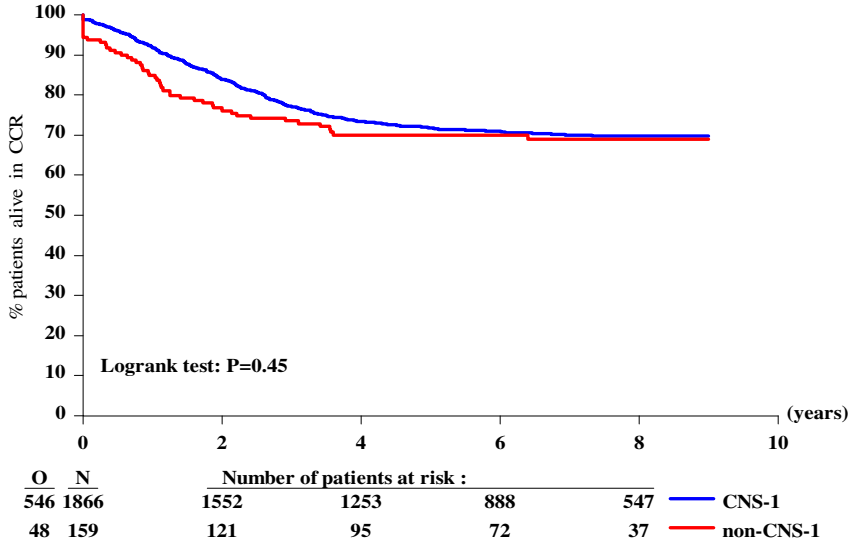

Fig. 2 – Event-free survival according to initial CNS status: CNS-1 versus non-CNS-1 (TLP+/CNS2/CNS3 status).

Cox multivariate analysis indicated that Medac E-coli asparaginase treatment, absence of VHR features and absence of NCI high risk features were each related to longer EFS and OS, whereas the presence of blasts at diagnosis in the CSF (TLP+, CNS-2 and CNS-3 versus CNS-1) had no prognostic value ($p = 0.33$ and $p = 0.81$, respectively, for EFS and OS) (Table 3). Pairwise comparisons according to each CNS status (TLP+ versus CNS-1, CNS-2 versus CNS-1, CNS-3 versus CNS-1) showed similar results (Table 3). Due to the initial correlation between CNS-3 status and bad prognostic features (NCI high risk, VHR), a drastic decrease of its prognostic importance was observed from univariate to multivariate analysis, especially regarding OS: estimated HR (CNS-3 versus CNS-1) was 1.93 versus 1.13 (Table 3).

The 8-year EFS rates (SE%) of TLP+ patients having 2 consecutive lumbar puncture with blasts (TLP++ group; $n = 21$), and therefore treated as CNS-3 patients, were lower, although not significantly ($p = 0.29$), than those of TLP+ not treated as CNS-3 patients ($n = 39$): 56.7% (12.5%) versus 74.1% (7.1%). The 8-year overall EFS rates (SE%) of CNS-2 patients having 2 consecutive lumbar puncture with blasts (CNS-2++ group; $n = 17$), and therefore treated as CNS-3 patients, were lower than those of CNS-2 patients not treated as CNS-3 patients ($n = 33$): 58.8% (11.9%) versus 77.3% (7.6%) (HR = 2.4; $p = 0.10$). In a Cox multivariate analysis, the EFS hazard rate was 4.64 times higher for CNS-2 patients having two consecutive lumbar puncture with blasts versus those without blasts on D3, after adjustment for NCI criteria ($p = 0.22$), VHR features ($p = 0.71$) and asparaginase treatment ($p = 0.003$).

Distribution of relapses according to CNS status group was studied. Out of 1994 patients who reached CR, a total of 71 isolated and 78 combined CNS relapses were reported. The 8-year overall disease-free survival (DFS) rate was 70.7% (SE 1.1%). The 8-year DFS rates (SE%) for patients in each CNS

Table 3 – Results of the Cox proportional hazards model regarding EFS and OS.

Variable	EFS				OS			
	HR	Lower limit	Upper limit	*p*-value	HR	Lower limit	Upper limit	*p*-value
Univariate analysis								
TLP+ versus CNS-1	1.08	0.67	1.72	0.76	1.14	0.64	2.02	0.67
CNS-2 s versus CNS-1	1.01	0.59	1.71	0.98	1.27	0.69	2.31	0.44
CNS-3 versus CNS-1	1.34	0.81	2.20	0.25	1.93	1.15	3.23	0.01
Multivariate analysis								
NCI risk group: HR versus SR	1.93	1.62	2.30	<.0001	2.17	1.74	2.71	<.0001
Initial VHR versus non-VHR	1.91	1.56	2.34	<.0001	2.70	2.15	3.41	<.0001
Asparaginase group: Medac E-Coli versus others	0.65	0.55	0.77	<.0001	0.62	0.51	0.76	<.0001
TLP+ versus CNS-1	0.77	0.48	1.23	0.27	0.70	0.39	1.25	0.23
CNS-2s versus CNS-1	0.98	0.58	1.67	0.94	1.18	0.65	2.16	0.58
CNS-3 versus CNS-1	0.89	0.54	1.47	0.64	1.13	0.67	1.91	0.64
Univariate analysis								
Non-CNS-1 versus CNS-1	1.13	0.84	1.51	0.43	1.40	1.01	1.96	0.047
Multivariate analysis								
NCI risk group: HR versus SR	1.93	1.62	2.29	<.0001	2.17	1.74	2.71	<.0001
Initial VHR versus non-VHR	1.91	1.56	2.33	<.0001	2.68	2.13	3.38	<.0001
Asparaginase group: Medac E-Coli versus others	0.65	0.55	0.77	<.0001	0.62	0.51	0.76	<.0001
Non-CNS-1 versus CNS-1	0.86	0.64	1.16	0.33	0.96	0.68	1.35	0.81

Table 4 – Outcome (CR rate, cumulative incidence at 8 years of isolated CNS relapse, of combined CNS, of both, of non-CNS relapse, of death in CR, and 8-year DFS rate), overall and according to initial CNS status.

At 8-years	All patients		CNS-1		TLP+		CNS-2		CNS-3	
	CI (%)	SE (CI) (%)	CI (%)	SE (CI) (%)	CI (%)	SE (CI) (%)	CI (%)	SE (CI) (%)	CI (%)	SE (CI) (%)
CR rate[a]	94.7	0.27	98.8	0.25	96.7	2.3	98.0	2.0	87.8	4.7
Isolated CNS	3.57	0.42	3.42	0.43	1.72	1.73	6.12	3.46	9.37	4.52
Combined CNS	4.04	0.45	4.14	0.48	1.72	1.73	4.08	2.86	2.33	2.33
Any-CNS	7.61	0.61	7.57	0.63	3.45	2.42	10.20	4.37	11.70	4.98
Non-CNS	18.59	0.91	18.72	0.95	23.69	5.89	12.66	4.91	11.63	4.95
Death in CR	3.10	0.39	3.18	0.41	1.72	1.72	4.39	3.09	0	0
DFS rate	70.7	1.06	70.54	1.10	71.1	6.2	72.8	6.5	76.7	6.5

[a] After induction/consolidation.

group were similar (Table 4). Overall, the DFS of patients with blasts at diagnosis in the CSF (TLP+, CNS-2 and CNS-3) was not significantly different ($p = 0.77$) from that of those with CNS-1 status.

The 8-year overall isolated CNS cumulative incidence was 3.6% (SE 0.4%). The 8-year isolated CNS relapse cumulative incidence (SE%) in each CNS group was as follows: 3.4% (0.4%) in CNS-1, 1.7% (1.7%) in TLP+, 6.1% (3.5%) in CNS-2 and 9.4% (4.5%) in CNS-3 (Table 4). Overall, the cumulative incidence of isolated CNS relapse in patients with CSF blasts at diagnosis was 5.3% and not significantly different ($p = 0.19$) from that found in CNS-1 patients.

The 8-year overall isolated or combined CNS relapse cumulative incidence was 7.6% (SE 0.6%). The 8-year isolated or combined CNS relapse incidence (SE%) in each CNS group was 7.6% (0.6%) in CNS-1, 3.5% (2.4%) in TLP+, 10.2% (4.4%) in CNS-2 and 11.7% (5.0%) in CNS-3 group (Table 4). Overall, the incidence of isolated or combined CNS relapse in patients with blasts at diagnosis was 8.0% (SE 2.2%) and not significantly different from that found in CNS-1 patients. Multivariate analysis indicated that only WBC count $< 25 \times 10^9$/L and Medac E-coli asparaginase treatment were each related to a significantly lower isolated or of any CNS relapse risk (Table 5). Neither the presence of CSF blasts at diagnosis (data not shown) nor the detailed CNS status was of prognostic significance (Table 5). Due to the high correlation between CNS-3 status and initial WBC count, comparison of CNS-3 versus CNS-1 adjusted by initial WBC count yielded an HR of 2.08, far lower than 2.98 provided in the univariate analysis (Table 5, isolated CNS relapse).

4. Discussion

The prognostic significance of blasts irrespective of leucocyte counts in the CSF remains controversial.[2,16–18] Our present report suggests that, in the EORTC study 58881, in which cranial radiotherapy was omitted for all patients, the presence of blasts irrespective of leucocyte counts in the spinal fluid of children with ALL has no prognostic significance for EFS and OS after adjustment for the main prognostic features (NCI risk criteria and VHR features).

Table 5 – Results of the Cox proportional hazards model regarding isolated CNS relapse rate and any-CNS relapse rate.

Variable	Isolated CNS relapse rate				Any-CNS relapse rate			
	HR	Lower limit	Upper limit	*p*-value	HR	Lower limit	Upper limit	*p*-value
Univariate analysis								
TLP+ versus CNS-1	0.53	0.07	3.79	0.52	0.48	0.12	1.94	0.30
CNS-2 s versus CNS-1	1.87	0.59	5.96	0.29	1.43	0.59	3.50	0.43
CNS-3 versus CNS-1	2.98	1.08	8.19	0.03	1.71	0.70	4.16	0.24
Multivariate analysis								
WBC: 25-<100 versus <25	2.65	1.57	4.50	0.0003	3.07	2.12	4.45	<.0001
WBC: ⩾100 versus <25	2.87	1.50	5.50	0.002	4.25	2.78	6.50	<.0001
Asparaginase group: Medac E-Coli versus others	0.43	0.27	0.68	0.0004	0.54	0.39	0.74	0.0002
TLP+ versus CNS-1	0.39	0.05	2.84	0.35	0.31	0.08	1.27	0.10
CNS-2 s versus CNS-1	1.86	0.58	5.96	0.29	1.34	0.55	3.27	0.53
CNS-3 versus CNS-1	2.08	0.74	5.83	0.16	1.04	0.42	2.57	0.93

We are aware of some drawbacks of our study.

(a) The CNS status classification used was derived from the conclusions of a workshop organised in 1985 in Rome, defining CNS leukaemia as the presence of more than five leucocytes/mm^{3}and the cytologic identification of any number of blasts in the CSF.[19] However, our classification was slightly different from the one later proposed by the investigators from the St Jude Children's Hospital.[20] The main difference was the threshold of the number of red blood cells required to consider the lumbar puncture as traumatic (100 RBCs instead of 10 RBCs). This difference could explain in part the distribution of patients within CNS-1, CNS-2 and TLP+ status groups in our study, somewhat different from the distribution reported by other groups.[2,3,8,17,18] Of note, the elective CNS-treatment was not significantly different for these 3 subgroups, except if the control lumbar puncture, performed 72 h after the initial one in CNS-2 and TLP+ patients still contained at least one blast cell.

(b) There was no central review of CSF cytospins in our study nor in the BFM 95. This may account in part for the lower incidence of CNS-2 patients, 2.5% in our study and 5.1% in the BFM 95, as compared to 21% in the DCLSG ALL-7 and -8 cohorts in which a central review of cytospins was mandatory, and to 31.6% and 20.4% in the St Jude Studies XIIIB and XV, respectively.[2,3,8,17] It is possible that some patients with only a few blasts in the CSF were mistakenly diagnosed as having CNS-1 status. This relatively small group of patients with unidentified CNS-2 status might have represented a selection bias.

(c) Finally, our low rate of TLP+ (2.9%) could possibly be attributed to the frequent administration of platelet transfusions before proceeding to the spinal tap in thrombocytopaenic patients (platelets counts below 50×10^9/L).[21]

In accordance with the results of other studies, TLP+, CNS-2 and CNS-3 patients considered together displayed significantly more unfavourable features. It came thus as no surprise that in the multivariate analysis, CNS status was not an independent prognostic factor of EFS.

The importance of systemic chemotherapy, and particularly HD MTX (5 g/m^2), is suggested by the favourable outcome of our CNS-3 patients, whose 8-year EFS rate, 68.3% (SE 6.1%), was similar to the 69.7% (SE 1.1%) achieved in our CNS-1 patients and better than the 50% (SE 8%) reported for CNS-3 patients in BFM 95 trial who had all received cranial radiotherapy, or the 43.2% (SE 23%) in the recent St Jude Study XV study, in which cranial radiotherapy was omitted in all patients.[2,8] Thirty per cent of our CNS-3 patients had VHR features as compared to 33% in the BFM 95 study. Worthy of note is that in the EORTC study 58881, all CNS-3 patients received either 9 courses of HD MTX (5 g/m^2) if VHR features were absent or 10 of these courses if VHR features were present, as compared to 4 courses (5 g/m^2) in the BFM 95 study, and four courses (5 g/m^2) in the St Jude Study XV study.[2,8] Our results are compatible with the hypothesis that HD MTX does contribute to the overall efficacy of ALL treatment, as was already suggested by the historical comparison of ALL-trials 81, 83, 86 of the BFM group, in which the administration of HD MTX resulted in better systemic control and better survival, when compared with intermediate dose methotrexate.[22] In a meta-analysis of 43 randomised trials, HD MTX reduced the haematologic relapse rate and improved EFS, but had only a marginal effect on the control of CNS leukaemia.[23] Another reason for our results in CNS-3 patients is probably due to intensive early intensification of intrathecal chemotherapy, with IT methotrexate administered every 3 d from the diagnosis until complete clearance of the blasts from the CSF.[24,25]

Considering the favourable results achieved in CNS-3 patients, it is disappointing to observe the dismal outcome of the patients with initial CNS-2 or TLP+ status and the persistent leukaemic blasts in the CSF 3 d after the first lumbar puncture. Although better than the 38% (SE 11.1%) EFS rate reported for equivalent TLP++ patients in the St Jude XII experience, the 56.7% (SE 7.9%) EFS in our study is worse than that of the CNS-3 cohort, in spite of their having received the same intensification of CNS-directed therapy. Pui and colleagues suggested that the particularly dismal outcome in these TLP++ patients proceeds from the high number of leukaemic

cells introduced into the CSF. For CNS-2 patients, whose CSF findings can be interpreted as an expression of 'minimal meningeal leukaemia', it is conceivable that the persistence of blast cells after an adequate early intrathecal therapy could be related to a diminished sensitivity to the treatment.

The incidence of isolated CNS relapses probably provides the best measure of the efficiency of CNS-directed therapy. For CNS-1 patients, this incidence was 3.4% and comparable to that of other contemporaneous studies: 4.1% in DCLSG ALL-7, 8.4% in DFCI ALL consortium protocol 87-01 and 1.6% in BFM 95.[2,17,18,26] In our study, the cumulative incidence of isolated CNS relapses in patients with blasts irrespective of CSF leucocyte counts at diagnosis was higher than but not significantly different from that of those with CNS 1 status. However, the incidence of isolated CNS relapse in our CNS-3 patients (9.3%), similar to the 11.1% in St Jude Study XV, compared unfavourably with equivalent patients in BFM 95 (3.4%) and DFCI ALL 87-01 (0%) studies, all of these having been treated with CNS-directed radiotherapy. Nevertheless, the small discrepancy in our CNS-3 patients between the cumulative incidence of isolated CNS relapses and that of isolated plus combined CNS relapses confirms that the intensity of systemic therapy, including the great number of HDMTX courses, affected predominantly the marrow rather than the CNS compartment in this group of patients. Indeed, our results are better than those of the BFM with regard to combined relapses with CNS involvement (2.4% versus 9.6%) although the cumulative incidence of isolated relapses was higher in our study.

Our results also confirm that L-asparaginase plays a role in preventing CNS relapses, whatever the CNS status of patients be in the EORTC 58 881 study. Riccardi and colleagues demonstrated in humans that the grade of asparagine depletion, within the CSF as an informative indicator of asparaginase effect, was dose and schedule dependent.[27] CSF asparagine levels inversely correlated with plasma L-asparaginase activity. These results were confirmed by several clinical trials showing that CSF asparagine levels were depleted in the majority of patients after conventional asparaginase therapy.[28,29] However, Mohgrabi and colleagues also recently observed a significantly higher CNS relapse rate for patients randomised to Erwiniase instead of E.-coli asparaginase (6% versus 1%, $p < 0.01$), when the two preparations were administrated at the same dose (25,000 IU/m^2 weekly $\times$ 20 doses), but exclusively in non-irradiated patients.[30] Our results, in relation with the shorter half-life of Erwinia preparation responsible of a lesser plasma activity, confirm that the type of asparaginase affects not only the event free survival and the risk of relapse as reported previously but also the type of relapse in non-irradiated patients.[12]

In conclusion, our study has shown that adjusting for other factors, the presence of blasts, whether or not associated with increased leucocyte counts in the CSF of children with ALL, had no prognostic significance in the EORTC 58881 study, and that CNS-involvement was not associated with an increased isolated CNS relapse rate. CNS-directed treatment and systemic chemotherapy in CNS-2 and TLP+ patients with two consecutive spinal taps containing blast cells require intensification. Our good results in CNS-3 patients indicate that a strategy without radiotherapy based on intensification of systemic therapy with high dose methotrexate is valuable for such patients, even if CNS-directed therapy in those patients needs intensification.

All these results were obtained with prednisolone during induction. There is evidence that dexamethasone has higher efficacy than prednisolone within the CNS compartment.[31] The substitution of dexamethasone for prednisolone might further improve the prevention of CNS relapses, just as the intensification of asparaginase therapy whose role in CSF therapy has indirectly been confirmed in our study. These two hypotheses are currently tested in EORTC 58951 trial.

Conflict of interest statement

None declared.

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REFERENCES

1. Pui CH, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. *Lancet Oncol* 2008;**9**:257–68.
2. Burger B, Zimmerman M, Mann G, et al. Diagnostic fluid examination in children with acute lymphoblastic leukemia: significance of low leucocyte counts with blasts or traumatic lumbar puncture. *J Clin Oncol* 2003;**21**:184–8.
3. Pui CH, Sandlund JT, Pei D, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. *Blood* 2004;**104**:2690–6.
4. Schriock EA, Schell MJ, Carter M, et al. Abnormal growth patterns and adult short stature in 115 long-term survivors of childhood leukemia. *J Clin Oncol* 1991;**9**:400–5.
5. Pui CH, Cheng C, Leung W, et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. *N Engl J Med* 2003;**349**:640–9.
6. Walter AW, Hancock ML, Pui CH, et al. Secondary brain tumours in children treated for acute lymphoblastic leukemia at St Jude Children's Research Hospital. *J Clin Oncol* 1998;**16**:3761–7.
7. Hilden JM, Dinndorf PA, Meerbaum SO, et al. CCG 1953: acute lymphoblastic leukemia in infants: analysis of prognostic factors: a report from the Children's Oncology Group. *Blood* 2006;**108**:441–51.
8. Pui CH, Campana D, Pai D, et al. Treating childhood acute lymphoblastic leukaemia without cranial irradiation. *N Engl J Med* 2009;**360**:2730–41.
9. Pui CH. Central nervous system disease in acute lymphoblastic leukemia: prophylaxis and treatment. *Hematology* 2006:142–6.
10. Vilmer E, Suciu S, Ferster A, et al. Long-term results of three randomized trials in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. *Leukemia* 2000;**14**:2257–66.
11. Langermann HJ, Henze G, Wulf M, Riehm H. Estimation of tumor cell mass in childhood acute lymphoblastic leukemia: prognostic significance and practical application. *Klin Padiatr* 1982;**194**:209–13.
12. Duval M, Suciu S, Ferster A, et al. Comparative toxicity and efficacy of E coli-asparaginase and Erwinia-asparaginase in childhood lymphoid malignancies: results of an EORTC-CLCG phase 3 randomized trial. *Blood* 2002;**8**:2734–9.
13. Millot F, Suciu S, Philippe N, et al. Value of high-dose Ara-C during the interval-therapy of a BFM-based protocol in increased risk children with acute lymphoblastic leukemia and lymphoblastic lymphoma: results of the EORTC 58881 randomized phase III trial. *J Clin Oncol* 2001;**19**:1935–42.
14. n Bosch J, Suciu S, Thyss A, et al. Value of intravenous 6-mercaptopurine during continuation treatment in childhood acute lymphoblastic leukemia and non-Hodgkin's lymphoma: final results of a randomized phase III trial (58881) of the EORTC CLG. *Leukemia* 2005;**19**:721–6.
15. Kalbfleisch JD, Prentice RL. *The survival analysis of failure time data*. 2nd ed. NJ (USA): Wiley Inter-Science; 2002. p. 462 [Wiley series in probabilities and statistics].
16. Pui CH. Toward optimal central nervous system-directed treatment in childhood acute lymphoblastic leukemia. *J Clin Oncol* 2003;**2**:179–81.
17. Maroeska D, Kamps w, van der Boes-van den Berg A, van Wering E, de Graaf S. Prognostic significance of Blasts in the cerebrospinal fluid without pleiocytosis or a traumatic lumbar puncture in children with acute lymphoblastic leukemia: experience of the Dutch Childhood Oncology Group. *J Clin Oncol* 2006;**24**:2332–6.
18. Gajjar A, Harrison P, Sandlund J, et al. Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. *Blood* 2000;**96**:3381–4.
19. Mastrangelo R, Poplack D, Bleyer A, et al. Report and recommendations of the Rome workshop concerning poor-prognosis acute lymphoblastic leukemia in children: biologic bases for staging, stratification and treatment. *Med Pediatr Oncol* 1986;**14**:191–4.
20. Mahmoud HH, Rivera GK, Hancock ML, et al. Low leucocytes counts with blast cells in cerebrospinal fluid with newly diagnosed acute lymphoblastic leukemia. *N Engl J Med* 1993;**329**:314–9.
21. Howard S, Gajjar A, Cheng C, et al. Risk factors for traumatic and bloody lumbar puncture in children with acute lymphoblastic leukemia. *JAMA* 2002;**288**:2001–7.
22. Dordelmann M, Reiter A, Zimmermann M, et al. Intermediate dose methotrexate is as effective as high-dose methotrexate in preventing isolated testicular relapse in childhood acute lymphoblastic leukemia. *J Ped Hematol Oncol* 1998;**20**:444–50.
23. Clarke M, Gaynon P, Hann I, et al. CNS-directed therapy for childhood acute lymphoblastic leukemia: childhood ALL Collaborative Group overview of 43 randomized trials. *J Clin Oncol* 2003;**21**:1798–809.
24. Conter V, Arico M, Valsechhi M, et al. Extended intrathecal methotrexate may replacecranial irradiation for prevention of CNS relapse in children with intermediate-risk acute lymphoblastic leukemia treated with Berlin–Frankfurt–Münster based intensive chemotherapy. *J Clin Oncol* 1995;**13**:2497–502.
25. Pui CH, Mahmoud H, Rivera K, et al. Early intensification of intrathecal chemotherapy virtually eliminates central nervous system relapse in children with acute lymphoblastic leukemia. *Blood* 1998;**2**:411–5.
26. LeClerc JM, Billet AL, Gelber RD, et al. Treatment of Childhood acute lymphoblastic leukemia: results of Dana-Farber ALL consortium protocol 87-01. *J Clin Oncol* 2002;**20**:237–46.
27. Ricardo R, Holcenberg JS, Glaubiger DL, et al. L-Asparaginase pharmacokinetics and asparaginase levels in cerebrospinal fluid of rhesus monkeys and humans. *Cancer Res* 1981;**41**:4554–8.
28. Woo MH, Hak LJ, Storm MC, et al. Cerebrospinal fluid asparagine concentrations after *Escherichia coli* Asparaginase in children with acute lymphoblastic leukemia. *J Clin Oncol* 1999;**17**:1568–73.
29. Gentili D, Conter V, Rizzari C, et al. L-Asparaginase depletion in plasma and cerebro-spinal fluid of children with acute lymphoblastic leukemia during subsequent exposures to Erwinia L-asparaginase. *Ann Oncol* 1996;**7**:725–30.
30. Mohgrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL consortium protocol 95-01 for children with acute lymphoblastic leukemia. *Blood* 2007;**109**:896–904.
31. Mitchell C, Richards S, Harrison C, Eden T. Long-term follow-up of the United Kingdom medical research coucil protocols for childhood acute lymphoblastic leukemia, 1980–2001. *Leukemia* 2010;**24**:406–18.