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Early morbidity and mortality in childhood acute lymphoblastic leukemia with very high white blood cell count

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Patients with white blood cell (WBC) count in peripheral blood $\geqslant 200 \times 10^9$/l at diagnosis of acute lymphoblastic leukemia (ALL) constitute 5–8% of all childhood ALL patients and are known to have significantly lower survival rates.[1,2] Hyperleukocytosis at diagnosis of childhood ALL is an oncological emergency associated with early morbidity and mortality related both to microcirculatory leukostasis and hyperviscosity and to severe metabolic and electrolyte derangements due to tumor lysis.[3,4] Since the introduction of urate oxidase, the risk of tumor lysis syndrome (TLS) has been markedly reduced.[5–8] However, clinicians may still reduce the dose of anticancer agents or delay the antileukemic therapy to avoid TLS, but the impact of such modifications on the risk of developing of early complications related to hyperleukocytosis and on survival is uncertain.

We performed a population-based multicenter study of 221 children aged 0–14.9 years with ALL and WBC $\geqslant 200 \times 10^9$/l at diagnosis treated in Denmark, Finland, Iceland, Lithuania, Norway or Sweden from January 1992 to October 2011. This constituted 92% of all 241 ALL patients with WBC $\geqslant 200 \times 10^9$/l during that period, and 6% of all 3985 newly diagnosed ALL patients.

The diagnosis of ALL was based on histo-/cytomorphology, immunophenotyping, karyotyping and molecular cytogenetics as previously reported.[9] For two infants and four non-infants, immunophenotype was lacking or ambiguous. Informed consent to antileukemic treatment was obtained according to the Declaration of Helsinki. The regional or national ethics committees approved the study.

Data were retrieved from the Nordic Society for Paediatric Hematology and Oncology (NOPHO) leukemia registry and from patient files. Morbidity and mortality within a month after admission was registered and analyzed in detail. Of 12 patients registered in the NOPHO leukemia registry with induction deaths, two occured 45 and 59 days after admission, and thus were not included into the present study as early deaths.

Overall, the clinical presentation and the pattern of leukostasis or hyperviscosity associated complications were similar to those previously reported[4,10–12] (Table 1). In total, 40% ($N = 92$) experienced one or more complications associated with hyperleukocytosis (Table 1 and Supplementary Figure 1). Their initial WBC was moderately higher than for patients without such complications (median (75% range): 396 (245–794) $\times 10^9$/l vs 317 (219–603) $\times 10^9$/l, ($P = 0.001$)). Thus, the absolute risk of complications increased 1.5-fold with every WBC increase of 100×10^9/l.

Ten patients (5%) died within the first month of treatment, of whom eight were older than one year at diagnosis and seven of these had T-ALL. Four patients died 13–27 days after admission because of neutropenic septic complications, whereas the remaining six patients died within 14 days from admission because of intracranial hemorrhage ($N = 5$) or massive intracranial infiltrates with secondary brain edema and herniation ($N = 1$). Coagulation disturbances were not demonstrated. Four of these latter six patients had initial WBC of 577, 768, 825 and 925×10^9/l and presented with severe central nervous system symptoms already at admission. Whereas the last two patients developed such symptoms on the third day after the rise in WBC from 305 to 625×10^9/l within 3 days on a prednisolone dose of 11.7 mg/m^2/24 h, or after a limited reduction in WBC from 395 to 291×10^9/l. Furthermore, in two of these six patients corticosteroids (CS) were not started (WBC 768×10^9/l) or were delayed (WBC 925×10^9/l) due to efforts to carry out leukapheresis.

In multivariate logistic regression analysis, only WBC (OR (95% CI): 1.004 (1.001–1.006), ($P = 0.007$)) and the presence of neurological symptoms at admission (OR (95% CI): 5.8 (1.3–25.2), ($P = 0.018$)) were independently and significantly associated with risk of early death, whereas neither gender, age, immunophenotype, leukemic karyotype and administration of antileukemic therapy within 24 h after admission versus later, nor hemoglobin at admission or administration of packed red blood cell transfusion when WBC was still $\geqslant 200 \times 10^9$/l were found to be of significance.

Initial therapy was heterogeneous and center-dependent as there was no common Nordic/Baltic tumor burden reducing strategy for patients with hyperleukocytosis (Figure 1 and Supplementary Figure 2). The majority (85%) of the patients were initially hydrated with $\geqslant 3000$ ml/m^2/24 h, and urine was alkalinized and allopurinol given to 89% and 97%, respectively, of those who did not receive urate oxidase. The urate oxidase was administered from 1 to 10 (median: 5) days. It was initiated before or on the same day as the administration of any antileukemic treatment for 96% of the 71 patients who received this treatment with available information on timing of the first dose. Administration of a CS prephase was optional for the seven infants and 159 older patients who were enrolled in NOPHO ALL-92, ALL-2000 or ALL-2008 clinical trials, and was mandatory both for 38 Nordic infants who were enrolled in the Interfant-99 or −06 clinical trials, and for the 17 Lithuanian patients who were treated according to BFM-based chemotherapy. Intrathecal MTX had to be initiated no later than on the first day of any other antileukemic therapy (Supplementary Table 1).

Indications for exchange transfusion or leukapheresis were according to local guidelines, and were performed in 24 and 12

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Table 1. Baseline characteristics of the patients and hyperleukocytosis related clinical symptoms and complications

	No. of patients (%)	*WBC $\times 10^9$/l Median (75%)*[a]	P	*Age, years Median (75%)*[a]	P
Infants[b]	48 (22)	454 (258–1058)		0.5 (0.2–0.8)	
Boys	20 (39)	476 (223–990)		0.5 (0.2–0.7)	
Girls	28 (61)	439 (258–1127)	0.73	0.4 (0.1–0.8)	0.34
BCP, ⩾1.0 years	49 (22)	306 (222–564)		5.7 (1.7–12.9)	
Boys	26 (51)	394 (221–602)		4.2 (1.8–14.0)	
Girls	23 (49)	284 (222–525)	0.12	7.1 (1.2–10.8)	0.78
T-ALL, ⩾1.0 years	120 (54)	340 (227–614)		7.9 (2.8–13.3)	
Boys	87 (72)	325 (230–577)		7.8 (2.7–13.4)	
Girls	33 (23)	424 (210–678)	0.12	7.9 (2.7–12.2)	0.77
Cytogenetic aberrations					
Normal karyotype	64 (29)	328 (230–677)		8.1 (2.0–13.4)	
11q23/*MLL*	44 (20)	490 (242–1022)		0.6 (0.3–9.1)	
t(9;22)[*BCR/ABL*]	5 (2)	284 (213–570)		6.4 (1.9–7.2)	
Hypodiploid	3 (1)	546 (256–556)		8.6 (0.2–8.8)	
t(12;21)	4 (2)	283 (225–514)		3.5 (1.9–12.1)	
t(1;19)	1 (0.5)	297		0.8	
Other	67 (30)	338 (225–621)		6.0 (1.2–13.5)	
Not available	34 (15)	321 (203–567)		6.5 (0.6–10.9)	
Neurological complications[c]					
Yes	33 (15)	530 (256–823)		8.7 (1.8–14.3)	
No	188 (85)	327 (225–624)	<0.001	5.3 (0.6–11.3)	0.007
Respiratory distress[d]					
Yes	15 (7)	620 (222–1400)		0.6 (0.1–8.6)	
No	206 (93)	336 (225–637)	0.006	6.1 (0.6–12.6)	0.001
Bleeding complications[e]					
Yes	43 (19)	420 (261–821)		9.3 (1.1–14.5)	
No	179 (81)	327 (223–666)	0.005	5.2 (0.5–11.2)	0.001
Severe complications requiring treatment at ICU					
Yes	24 (11)	522 (248–914)		4.1 (0.2–13.1)	
No	197 (89)	334 (225–625)	0.002	6.0 (0.6–12.3)	0.62
Dialysis					
Yes	11 (5)	310 (202–528)		6.5 (3.1–12.6)	
No	211 (95)	357 (227–714)	0.28	5.8 (0.6–12.3)	0.34
Any complication					
Yes	92 (42)	396 (245–794)		6.1 (0.7–13.3)	
No	130 (58)	317 (219–603)	0.001	5.3 (0.5–10.9)	0.03
Renal infiltrations	10 (5)	398 (254–690)		3.0 (0.3–11.6)	
Skin infiltrations	7 (3)	446 (230–720)		0.6 (0.0–12.7)	
Priapism	2 (0.9)	621; 394		3.9; 6.4	

Abbreviations: BCP, B-cell precursor acute lymphoblastic leukemia; ICU, intensive care unit; WBC, white blood cell count. Complications were present at or developed within three days from admission. Hyperleukocytosis related neurological symptoms, respiratory distress or bleeding complications assessed as grade 2–4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE)[14] were registered. WBC count = x10^9/l in peripheral blood at admission. *P*-value = determined after comparison by Mann–Whitney *U*-test; hypodiploid karyotype = modal chromosome number <45; other = non-stratifying cytogenetic aberrations. [a]Median, minimal and maximal values are provided when number of cases is less than eight. [b]For two infants and four non-infant ALL patients immunophenotype was unavailable or ambiguous. [c]Severe headache, dizziness, irritability, consciousness disturbances, peripheral nerve palsies and seizures. [d]Dyspnea or adults respiratory distress syndrome. [e]Epistaxis, metrorrhagia, gastrointestinal or intracranial bleeding.

patients, respectively. Such mechanical cytoreduction was given either as the first tumor reducing treatment modality ($N=15$) or concomitantly with administration of CS and/or intrathecal MTX ($N=21$). The median (75% range) absolute and relative reduction in WBC per procedure for exchange transfusions was 298 (81–674) $\times 10^9$/l and 57% (26–82%), respectively, and for leukapheresis it was 165 (67–337) $\times 10^9$/l and 48% (26–68%), respectively ($P=0.11$). Antileukemic therapy was delayed more often for the patients with mechanical cytoreduction as first treatment modality ($N=15$) compared with all the remaining patients ($N=191$); the median (range) time to administration was: 1.5 (1.0–3.0) vs 1.0 (0–6.0) days, respectively, ($P=0.009$).

TLS defined according to classification proposed by Cairo and Bishop[13] developed in 27 patients (12%): 5 infants, 1 non-infant B-cell precursor (BCP) and 21 T-ALL patients. TLS was present at admission ($N=8$) or developed within 3 days after initiation of any antileukemic therapy ($N=19$). Four out of the latter 19 patients developed TLS after only intrathecal MTX had been administered. Patients who developed TLS had significantly higher initial uric acid level than those who did not, both within the cohort of all patients (median: 652 vs 460 μmol/l; $P<0.001$) and specifically within the T-ALL group (median: 661 vs 420 μmol/l; $P<0.001$). After initiation of antileukemic therapy, TLS developed in 16 of 118 patients (14%) who received only CS compared with 2 of 60 patients (3%) who recieved both CS and upfront urate oxidase ($P=0.03$). Eleven patients (5%) were dialyzed (ten patients with T-ALL and one non-infant BCP patient). No patients died owing to TLS or its treatment.

The initial CS dose (calculated as a prednisolone equivalent dose) did not differ significantly for patients who developed TLS vs those who did not ($P=0.19$). Furthermore, none of the patients who received an initial prednisolone equivalent dose of 60 mg/m^2/24 h as prephase ($N=11$, median WBC 303×10^9/l) or full induction therapy including prednisolone, vincristine and doxorubicin ($N=6$, median WBC 375×10^9/l) developed TLS. Urate oxidase had been given to 4 out of these 17 patients. Multivariate logistic regression analysis revealed uric acid level at admission to be the only significant risk factor for TLS (OR (95% CI): 1.005 (1.001–1.008), $P=0.009$), whereas neither gender, age, initial WBC or lactate dehydrogenase, time to start of antileukemic

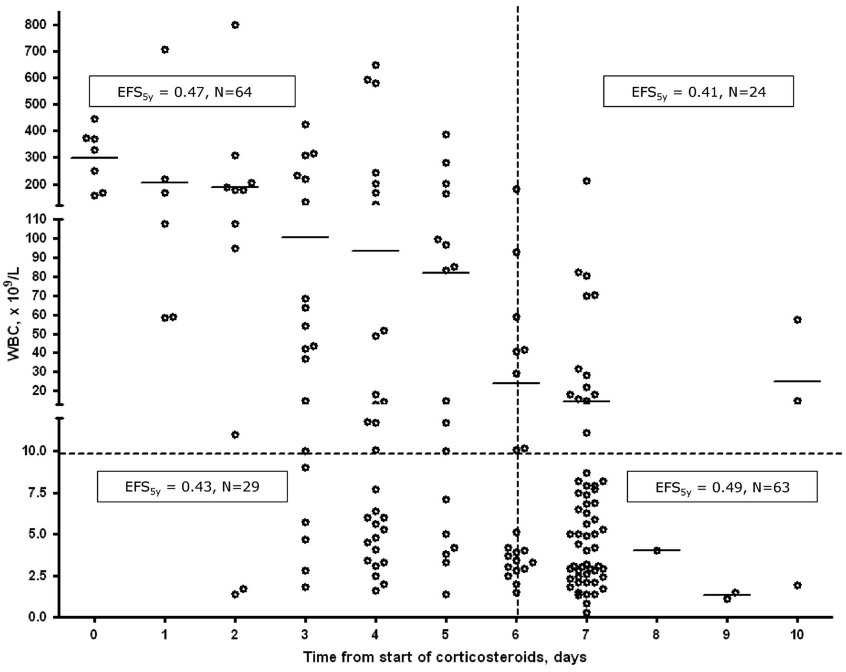

Figure 1. Distribution of white blood cell count in peripheral blood on the day of introduction of induction with vincristine and doxorubicin. Each dot represents one patient. Horizontal bars represent median WBC values. Dashed lines mark median WBC at the day of introduction of induction and median duration of CS prephase. WBC count ($\times 10^9$/l); pEFS$_{5y}$ = projected 5-year event-free survival within subsets.

therapy, initial CS dose nor mechanical cytoreduction procedures were significantly associated with the risk of TLS.

There was a borderline significant improval in the 5-year probability of event-free survival being $0.35 + / - 0.05$ for the 84 patients diagnosed before 2002 and $0.51 + / - 0.05$ for the 137 patients diagnosed in the latter period ($P = 0.07$). Cox multivariate regression analysis that explored gender, age, immunophenotype, karyotype, use of mechanical cytoreduction procedures, administration of a CS prephase, and development of TLS, found only BCP phenotype to be associated with an inferior disease-free survival (HR (95% CI) 1.9 (1.2–3.1); $P = 0.009$).

Based on these findings, current Nordic/Baltic guidelines for management of hyperleukocytosis $\geqslant 100 \times 10^9$/l and high risk for TLS recommend initiation of full induction with dexamethasone 10 mg/m^2/24 h, vincristine and doxorubicin within 24 h and as soon as all required diagnostic samples have been obtained and urate oxidase given (Supplementary Guidelines). For patients with metabolic derangements or clinical symptoms compatible with TLS at admission, to avoid TLS-associated complications, a prephase of prednisolone at a dose of 20 mg/m^2/24 h is recommended with rapid dose increments to full induction therapy within 48–72 h. Uric acid level are to be monitored at 8 h intervals and urate oxidase re-administered whenever urate level exceeds 100 μmol/l. The efficacy of this approach will be monitored prospectively.

In summary, this study supports that the main risk factors for TLS are T-cell immunophenotype and an increased level of uric acid at diagnosis, and that with use of contemporary supportive care and urate oxidase, the complications directly associated with hyperleukocytosis pose a significantly greater risk to the patients than do TLS. In addition, reduced initial chemotherapy doses or delaying antileukemic therapy (for example, due to mechanical cytoreduction as first treatment modality) may contribute to worsening of life-threatening leukostasis and risk of early deaths.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

GV, AHS, AÅ, BL and KS designed the study; GV, MH, OGJ, BL, AHS, MS, MT and AÅ collected data; GV, MH, TZ and KS analyzed data; GV, LR and KS wrote the manuscript. All the authors revised the manuscript and gave their final approval.

G Vaitkeviciene[1,2], M Heyman[3], OG Jonsson[4], B Lausen[2], A Harila-Saari[5], M Stenmarker[6], M Taskinen[7], T Zvirblis[8], A Åsberg[9], L Groth-Pedersen[2], L Rageliene[1] and K Schmiegelow[2,10]
[1]*Clinic of Children's Diseases, Faculty of Medicine, Vilnius University, Vilnius, Lithuania;*
[2]*Department for Paediatric and Adolescent Medicine, Juliane Marrie Centre, University Hospital Rigshospitalet, Copenhagen, Denmark;*
[3]*Department of Paediatric Oncology, Astrid Lindgrens Childrens Hospital, Stockholm, Sweden;*
[4]*Department of Paediatrics, University Hospital Rigshospitalet, Reykjavik, Iceland;*
[5]*Oulu University Hospital, Oulu, Finland;*
[6]*Department of Paediatrics, Ryhov County Hospital, Jonkoping, Sweden;*
[7]*Children's Hospital, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland;*
[8]*Hematology, Oncology and Transfusion Medicine Center, Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania;*
[9]*Paediatric Department, St Olav's University Hospital, Trondheim, Norway and*
[10]*Department of Health Sciences, University of Copenhagen, Copenhagen, Denmark*
E-mail: kschmiegelow@rh.dk

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Gossypol overcomes stroma-mediated resistance to the BCL2 inhibitor ABT-737 in chronic lymphocytic leukemia cells *ex vivo*

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Chronic lymphocytic leukemia (CLL) is characterized by the deregulated accumulation and persistence of B lymphocytes in the blood. Although the exact causes of CLL are unknown, the evasion of apoptosis through the aberrant expression of B-cell lymphoma 2 (BCL2)-family proteins is a common feature.[1] A class of compounds, termed Bcl-2 homology domain 3 (BH3) mimetics, has been developed to directly inhibit BCL2 proteins and selectively kill tumor cells. To date, the most successful of these compounds are the BCL2/BCLXL inhibitors ABT-737[2] and ABT-263 (navitoclax),[3], as well as the BCL2-specific inhibitor ABT-199.[4] Results from early clinical trials with navitoclax have demonstrated single-agent efficacy in patients with relapsed or refractory CLL.[5] However, there was heterogeneity in response rates between patients and dose-limiting toxicities, including thrombocytopenia and neutropenia, which prevented further dose escalation. In addition, CLL cells residing within various microenvironments (for example, lymph nodes and bone marrow) are resistant to BCL2 inhibitors. This resistance results from the upregulation of additional BCL2 proteins, such as BCLXL, MCL1 and BFL1, the latter two of which are not inhibited by navitoclax, and therefore protect the leukemia cells from apoptosis.[6] Additional drugs are needed to enhance the efficacy of navitoclax. Here, we demonstrate that gossypol overcomes stroma-mediated resistance to ABT-737 without enhancing the sensitivity of normal lymphocytes and platelets.

The BH3-only protein, NOXA, is a potent inhibitor of MCL1 and BFL1,[7] but has recently been recognized to inhibit BCLXL with lower affinity.[8] Therefore, compounds which induce NOXA may inhibit MCL1, BFL1 and BCLXL, thus overcoming the resistance to navitoclax. We previously reported that six putative BH3 mimetics do not directly inhibit BCL2 in cells, but instead activate the integrated stress response, and induce NOXA.[9] Of these six compounds, gossypol has advanced into clinical trials in a racemically purified form (AT-101).[10] We hypothesized that gossypol, through the induction of NOXA, would sensitize CLL cells to ABT-737.

CLL cells from consented patients were incubated *ex vivo* with up to 10 μM ABT-737, which is comparable to the peak plasma concentration of navitoclax in a Phase I trial.[5] Apoptosis, as assessed by chromatin condensation, (or caspase cleavage of poly (ADP-ribose) polymerase; data not shown) was induced within 6 h as previously noted.[9] The CLL samples were highly sensitive to ABT-737, as a single agent, with most cells undergoing apoptosis between 10 and 100 nM (Figure 1a). Gossypol alone (5–20 μM) induced NOXA, but had minimal impact on apoptosis within this time frame. Importantly, gossypol sensitized the cells to ABT-737, with most cells now undergoing apoptosis between 1 and 10 nM.

Coculture of CLL cells on the stromal cells can mimic the protective microenvironment seen in the patients, and promote resistance to ABT-737 and navitoclax. Different stroma have variously been reported to increase the expression of MCL1, BFL1 and BCLXL.[6,11] We hypothesized that gossypol might overcome stroma-mediated resistance to ABT-737 by inducing NOXA. We used a coculture system, whereby CLL cells were incubated with fibroblasts expressing CD154 (CD40L).[12] Following 24-h coculture, BCLXL was markedly increased in all the CLL samples; there was a variable increase in BFL1 and no significant increase in MCL1 (Figure 1b). BCL2 expression was unchanged. In agreement with a previous report,[6] we observed at least 100-fold resistance, with most cells requiring 1–10 μM ABT-737 to induce apoptosis (Figure 1c). Importantly, we found that gossypol resensitized the CLL cells to ABT-737 in a concentration-dependent manner, with apoptosis again occurring at 10–100 nM ABT-737.

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