

## ORIGINAL ARTICLE

## High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies

Goda Vaitkevičienė<sup>1,2</sup>, Erik Forestier<sup>3</sup>, Marit Hellebostad<sup>4</sup>, Mats Heyman<sup>5</sup>, Olafur G. Jonsson<sup>6</sup>, Päivi M. Lähteenmäki<sup>7</sup>, Susanne Rosthøj<sup>8</sup>, Stefan Söderhäll<sup>5</sup>, Kjeld Schmiegelow<sup>2,9</sup> On behalf of the Nordic Society of Paediatric Haematology and Oncology (NOPHO)

<sup>1</sup>Centre for Paediatric Oncology and Haematology, University Childrens Hospital, Vilnius, Lithuania; <sup>2</sup>Department of Paediatrics, University Hospital Rigshospitalet, Copenhagen, Denmark; <sup>3</sup>Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden; <sup>4</sup>Department of Paediatrics, Ullevål University Hospital, Oslo, Norway; <sup>5</sup>Department of Paediatric Oncology, Astrid Lindgrens Childrens Hospital, Stockholm, Sweden; <sup>6</sup>Department of Paediatrics, University Hospital, Reykjavik, Iceland; <sup>7</sup>Department of Paediatrics, Turku University Hospital, Turku, Finland; <sup>8</sup>Department of Biostatistics, University of Copenhagen; <sup>9</sup>The Institute of Gynaecology, Obstetrics, and Paediatrics, The Faculty of Medicine, University of Copenhagen, Copenhagen, Denmark

### Abstract

Prognostic impact of peripheral blood white blood cell count (WBC) at the diagnosis of childhood acute lymphoblastic leukaemia (ALL) was evaluated in a population-based consecutive series of 2666 children aged 1–15 treated for ALL between 1992 and 2008 in the five Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). Ten-year event-free (pEFS<sub>10y</sub>) survival and overall (pOS<sub>10y</sub>) survival were  $0.75 \pm 0.01$  and  $0.85 \pm 0.01$ , respectively. Although treatment intensity was determined by WBC, non-remission and relapsed patients still had significantly higher WBC than those in remission for B-cell precursor (BCP) (median WBC: 24.8 vs. 14.0 vs.  $8.3 \times 10^9/L$ ,  $P < 0.001$ ), but not for T-lineage (T-ALL) (median WBC: 127.8 vs. 113.0 vs.  $86.8 \times 10^9/L$ ,  $P = 0.22$ ). pEFS was inversely related to WBC for BCP ( $P < 0.001$ ), but not for T-ALL. WBC was not associated with risk of event for BCP or T-ALL for patients with minimal residual disease at the end of induction (MRD<sub>d29</sub>)  $< 10^{-3}$ . In contrast, for MRD<sub>d29</sub>  $\geq 10^{-3}$  and  $< 5\%$  leukaemic blasts in bone marrow at day 29, the pEFS<sub>5y</sub> for WBC  $< 100.0$  ( $N = 152$ ) vs.  $\geq 100.0$  ( $N = 19$ ) was 0.76 vs. 0.50 ( $P = 0.001$ ). That was the case both for BCP (pEFS<sub>5y</sub> 0.76 vs. 0.58) and for T-ALL (pEFS<sub>5y</sub> 0.71 vs. 0.38). Whether the inferior EFS for the subset of patients with high WBC and slow initial response to treatment reflects rare or overlooked cytogenetic aberrations as well as the factors that determine WBC levels at diagnosis awaits exploration.

**Key words** white blood cell; acute lymphoblastic leukaemia; children; survival; prognostic factors; minimal residual disease

**Correspondence** Professor Kjeld Schmiegelow, The Pediatric Clinics, The Juliane Marie Centre, the University Hospital Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark. Tel: +45 3545 1357; Fax: +45 3545 4524; e-mail: kschmiegelow@rh.dk

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Historically, the white blood cell count in peripheral blood (WBC) at diagnosis has been one of the strongest independent predictors of induction failure, resistant disease and risk of relapse in childhood acute lymphoblastic leukaemia (ALL) and has accordingly been used for treatment stratification (1–6). However, the groups with high or low WBC are biologically heterogeneous, because they include different proportions of B-cell

precursor (BCP) and T-lineage ALL (T-ALL) and many cytogenetic subsets with different prognostic impact (7). As treatment has been intensified, new prognostic factors have emerged, including prognostic karyotypes (8, 9) and early assessment of minimal residual disease (MRD) (10–13). Many large international study groups currently use these as the main parameters for treatment stratification, while WBC at diagnosis has tended to lose its

significance. Because the overall survival rates for childhood ALL now reach 85%, future treatment protocols should include a strategy for identification of the lower risk subset among the patients previously classified to have higher risk ALL to treat these patients with regimens associated with a low risk of short- and long-term toxicity. The role of WBC in this strategy is uncertain. The purpose of this retrospective study of 2666 Nordic children with ALL was to explore in detail to which extent WBC as a surrogate marker of both the leukaemic clone and host characteristics is an independent predictor of risk of relapse in contemporary ALL protocols when adjusting for other prognostic features such as immunophenotype, karyotype, extramedullary disease and the early response to therapy measured as the level of MRD.

### Patients and methods

From January 1992 to January 2008, 2666 children aged 1.0–14.9 were diagnosed with BCP or T-ALL and enrolled on NOPHO ALL-92 and ALL-2000 protocols in the Nordic countries (Denmark, Sweden, Norway, Finland and Iceland). Twenty-eight patients were excluded from the study because of missing ( $n = 9$ ) or ambiguous ( $n = 19$ ) data on immunophenotype. The remaining 1443 boys and 1195 girls with 2371 BCP and 267 T-ALL constitute this study cohort, of which 1627 patients were treated according to the NOPHO ALL-92 protocol (1992–2001) and 1011 according to the ALL-2000 protocol (2002–2008). Thirty patients on the ALL-92 protocol and 24 on the ALL-2000 protocol had Down syndrome. They were eligible for treatment according to the NOPHO protocols and are included in this study but analysed separately.

The diagnosis of ALL was based on morphologic evaluation of bone marrow smears in combination with immunophenotyping with panels of monoclonal antibodies directed towards lineage-associated antigens according to well-established criteria (14). Only G-band karyotyping was mandatory in the ALL-92 protocol, but the ALL-2000 protocol also required directed analysis by fluorescent *in-situ* hybridisation and/or reverse transcriptase polymerase chain reaction (PCR) for translocations  $t(9;22)(q34;q11)[BCR-ABL]$  or  $t(1;19)(q23;p13)[E2A-PBX1]$  and for  $11q23/MLL$  aberrations. Furthermore, many leukaemic samples have been explored by comparative genomic hybridisation, spectral karyotyping and DNA index by flow cytometry, and nearly all patients have in recent years been explored for the  $t(12;21)[ETV6-RUNX1]$  translocation, although the presence of this translocation did not influence the treatment stratification (15). All cytogenetic results are reviewed annually by the NOPHO cytogenetic working group and described according to ISCN 1995 (16).

Central nervous system (CNS) disease at diagnosis was defined as an increased number of mononuclear cells ( $\geq 5 \times 10^6/L$ ) with leukaemic blasts in the diagnostic spinal tap and/or peripheral nerve palsy or confirmation of CNS disease on imaging. Testicular leukaemia was defined as swelling of testicles with leukaemic masses confirmed by ultrasonography and/or biopsy.

MRD was available for few patients in the ALL-92 study and was not used for treatment stratification in that protocol. In the ALL-2000 study, the goal of MRD measurement was to examine the feasibility of non-centralised MRD monitoring by flow cytometry and PCR late during induction at day 29 (MRD<sub>d29</sub>) but was not used for treatment stratification, except for MRD levels  $\geq 10^{-3}$  after 3 months of antileukaemic therapy being an optional indication for risk group upgrading which included stem cell transplantation (SCT). In total, MRD<sub>d29</sub> was performed by flow cytometry (FC-MRD) and/or PCR-based techniques for Ig and TCR gene rearrangements (PCR-MRD) in 752 cases (682 BCP and 70 T-ALL). FC-MRD<sub>d29</sub> was more predictive than PCR-MRD<sub>d29</sub> for BCP, as was PCR-MRD<sub>d29</sub> for T-lineage ALL, and these methods have been implemented in the current ALL-2008 protocol (17). Thus, to classify patients in this study according to MRD<sub>d29</sub>, we used FC-MRD<sub>d29</sub> results for BCP ALL or PCR-MRD<sub>d29</sub> if FC-MRD<sub>d29</sub> was unavailable (516 and 166 cases, respectively), and for T-lineage ALL we used PCR-MRD<sub>d29</sub> results or FC-MRD<sub>d29</sub> if PCR-MRD data were unavailable (50 and 20 cases, respectively).

The NOPHO ALL-92 and ALL-2000 protocols were approved by the regional or national ethics committees, and informed consent was obtained according to the Declaration of Helsinki.

### Risk grouping and treatment

#### NOPHO ALL-92 and ALL-2000 risk grouping

Details of the NOPHO ALL-92 and ALL-2000 protocols have been described in previous publications (6, 17–19). In ALL-92, the risk group assignment was based on age and white blood cell count (WBC) at diagnosis (standard risk, SR: age 2.0–9.9 and  $WBC < 10.0 \times 10^9/L$ ; intermediate risk, IR: age 1.0–1.9 or  $\geq 10.0$  and/or  $WBC 10.0–49.9 \times 10^9/L$ ; higher risk [i.e. high risk (HR) or very high risk (VHR)]:  $WBC \geq 50.0 \times 10^9/L$  and the presence of higher risk features: T-lineage ALL, the presence of CNS or testicular involvement, translocations  $t(9;22)(q34;q11)$  or  $t(4;11)(q21;q23)$ , lymphomatous leukaemia or mediastinal lymphoma and/or a poor treatment response (M3 BM at day 15 or M2/M3 at day 29) (6). The risk grouping was unchanged in ALL-2000 except that (i) all children aged 1.0–9.9 were eligible to the SR-arm, if their WBC was

<  $10.0 \times 10^9/L$ , and they had no HR group features, (ii) t(1;19)(q23;p13), hypodiploidy (<45 chromosomes), and all *MLL* rearrangements were allocated to higher-risk groups and (iii) it was optional to offer SCT in first remission to patients with MRD levels  $\geq 10^{-3}$  after 3 months of antileukaemic therapy. Cranial radiotherapy was restricted to the VHR patients older than 5 yr.

### NOPHO ALL-92 and ALL-2000 therapy strategy

Details of the NOPHO ALL-92 and ALL-2000 protocols are given in Tables S1 and S2.

**Induction therapy:** In ALL-92, all patients received prednisolone ( $60 \text{ mg/m}^2/\text{d}$  on days 1–36), weekly vincristine (VCR) ( $2.0 \text{ mg/m}^2$  six times), doxorubicin [ $40 \text{ mg/m}^2$  three times (SR and IR) or four times (HR)], Erwinia asparaginase ( $30,000 \text{ IU/m}^2$  daily on days 37–46) and intrathecal (i.t.) methotrexate (MTX) four times. ALL-2000 induction therapy was identical to that of the ALL-92 protocol except that (i) one dose less of doxorubicin was given, (ii) the maximum dose of VCR was set to 2.5 mg, and (iii) Erwinase was substituted with *E. coli* asparaginase ( $6,500 \text{ IU/m}^2$  at 3-d intervals, four times).

Early intensification consisting of cyclophosphamide, oral 6-mercaptopurine (6-MP), low-dose cytarabine and i.t. MTX was given to IR and HR patients immediately after the induction phase.

**Consolidation therapy:** In ALL-92, the therapy included high-dose MTX (HD-MTX) at  $5 \text{ g/m}^2/24 \text{ h}$  for SR and IR with i.t. MTX and leucovorin rescue, whereas HR and VHR patients received HD-MTX  $8 \text{ g/m}^2/24 \text{ h}$  alternating with high-dose cytarabine ( $12 \text{ g/m}^2$ ) with 2-month intervening periods of oral MTX and 6-MP with two VCR/prednisolone reinductions per period (20). In ALL-2000, SR and IR patients received three HD-MTX courses alternating with low-dose cytarabine blocks with concomitant 6-MP, whereas HD-MTX consolidation therapy for higher-risk patients was identical to that of the ALL-92 protocol. **Delayed intensification** in both ALL-92 and ALL-2000 was given to IR and higher-risk patients and consisted of oral dexamethasone, weekly VCR, weekly anthracycline and asparaginase (Erwinia asparaginase in ALL-92 and *E. coli* asparaginase in ALL-2000), followed by cyclophosphamide, low-dose cytarabine and 6-thioguanine (6, 17).

Classical oral 6-MP/MTX *maintenance therapy* continued until 2 yr (for IR and HR in ALL-92 and for HR and VHR in ALL-2000) or 2.5 yr (for SR in ALL-92 and for SR and IR in ALL-2000) after diagnosis. During the first year of maintenance therapy, SR or IR-ALL received in addition alternate pulses of VCR and corticosteroids and HD-MTX. HR and VHR received reinductions of VCR and corticosteroids. In ALL-92, patients with VHR ALL (and all Finish patients with

HR ALL) received cyclic LSA<sub>2</sub>L<sub>2</sub> maintenance therapy instead of oral 6-MP/MTX maintenance therapy, while in ALL-2000 LSA<sub>2</sub>L<sub>2</sub> was given two (HR) or three times (VHR) prior to the start of oral MTX/6MP maintenance therapy, or until SCT could be performed (VHR ALL who fulfilled criteria for SCT) (6, 17).

A subset of VHR patients, who in addition were aged 5 and older, was offered *cranial irradiation* in the ALL-92 ( $N = 158$ ) and ALL-2000 ( $N = 128$ ) protocols.

**Haematopoietic SCT:** In total, 57 patients (3.5%) and 62 patients (6.1%) received SCT in first remission in ALL-92 and ALL-2000 protocols, respectively. There were no uniform Nordic criteria for SCT in ALL-92. In ALL-2000, SCT was indicated in case of  $\text{WBC} \geq 200.0 \times 10^9/L$  at diagnosis, hypodiploidy (<34 chromosomes) and 11q23/*MLL* rearrangement and optional if  $\text{MRD} \geq 10^{-3}$  after 3 months of therapy.

### Statistical analysis

Survival analyses were performed with a basic time scale defined by the date of diagnosis. The projected duration of event-free survival (pEFS) was calculated from diagnosis until the date of assessment of relapse, death in first complete remission (1CR) or the development of a second malignancy (whichever first) or the last known follow-up for event-free survivors. The patients who did not achieve complete remission (induction failure or resistant disease) were counted as an event at a time of zero. Non-parametric methods were applied to compare the distribution of parameters between subgroups (21). The chi-square test was used for the comparison of categorised variables, and the Mann–Whitney *U*-test was used for continuous variables. Two-sided *P*-values <0.05 were regarded as significant. The Kaplan–Meier method was applied for the estimation of remission duration and for the generation of survival curves, and the log rank test was used to test the prognostic differences among subgroups (22). Cox proportional hazards regression analysis was performed to identify independent prognostic factors for differences in outcome (23, 24). Treatment results were evaluated both for all the patients in the cohort and for the patients bearing none of the HR factors that were used for treatment stratification in the NOPHO ALL-92 and 2000 protocols. The latter were defined as CNS or testicular involvement, mediastinal mass, lymphomatous disease or HR cytogenetic aberrations (translocations t(9;22)[*BCR/ABL*] or t(1;19)(q23;p13), 11q23/*MLL* aberration or a hypodiploid karyotype (<45 chromosomes)). The impact of WBC on prognosis was analysed with respect to event-free survival (pEFS). All data were analysed using the statistical package for the social sciences (SPSS) software for Windows, version 15.0 (SPSS, Chicago, IL, USA).

The status of each patient in the NOPHO leukaemia registry is updated at least annually. Within 10 yr from the diagnosis of ALL (median: 6.7 yr), 18 patients from the ALL-92 cohort were lost to follow up in the NOPHO ALL registry because of emigration outside the Nordic countries ( $N = 3$ , median: 2.8 yr), change of Nordic address ( $N = 4$ , median: 5.2 yr), transfer to an adult department ( $N = 5$ , median: 6.8 yr) and cessation of clinical control or not-otherwise-specified ( $N = 6$ , median: 7.9 yr). Similarly, a total of eight patients from the ALL-2000 cohort were lost to follow up in the NOPHO ALL registry protocol at a median of 2.5 yr from diagnosis.

## Results

The median WBC at diagnosis for the 2371 BCP (89.9%) and 267 T-ALL cases (10.1%) was  $8.6 \times 10^9/L$  (75% range:  $2.3\text{--}56.5 \times 10^9/L$ ) and  $86.8 \times 10^9/L$  (75% range:  $8.8\text{--}394.5 \times 10^9/L$ ), respectively, with slightly higher WBC among boys than girls both within the BCP and T-ALL subsets (Table 1). Ninety seven per cent of patients with a WBC  $< 10.0 \times 10^9/L$  at diagnosis had BCP ALL, whereas the fraction of BCP and T-ALL among those with WBC  $\geq 100.0 \times 10^9/L$  was almost equal (53% and 47%, respectively) (Table 1).

## Cytogenetic aberrations

An informative normal or aberrant karyotype was recorded in 2323 patients (88.1%). The WBC at diagno-

sis differed significantly between the cytogenetic subgroups (Table 1). Patients with unfavourable cytogenetics [ $N = 89$ ; 11q23/*MLL* aberrations, translocation t(9;22) [*BCR/ABL*] or hypodiploidy (modal chromosome number  $< 45$ )] comprised only 2.6% of the BCP cases with WBC  $< 50.0 \times 10^9/L$ , but 11%, 18% and 30% of the BCP patients with WBC  $\geq 50.0$ ,  $\geq 100.0$  or  $\geq 200.0 \times 10^9/L$ , respectively. The 1039 cases with t(12;21)[*ETV6/RUNX1*] translocation or a high-hyperdiploid karyotype (HeH, modal chromosome number  $> 50$ ) together comprised 46% of BCP cases with WBC  $< 50.0 \times 10^9/L$  but only 7.5% of those with WBC  $\geq 200.0 \times 10^9/L$ . Except for the cases with 11q23/*MLL* aberrations, the WBC was inversely correlated with the age at diagnosis for all cytogenetic subsets and that was the case both for girls ( $r_s = -0.19$ ,  $P < 0.001$ ) and for boys ( $r_s = -0.15$ ,  $P < 0.001$ ) and was most significant for the patients with translocation t(12;21) and HeH karyotype ( $r_s = -0.27$  ( $P = 0.01$ ) and  $r_s = -0.28$  ( $P = 0.01$ ), respectively) ( $P$ -values after Bonferroni correction) (Table 1). The latter two subsets are characterised by a prominent incidence peak between 1 and 7 yr of age, and together they encompass 78% of all BCP ALL cases with an aberrant karyotype in this age group (7).

## Extramedullary disease

The WBC for 67 patients with CNS disease at diagnosis (2.6% of all patients) was higher than for those without

**Table 1** White blood cell count in peripheral blood (WBC) at diagnosis in T-lineage (T-ALL) and cytogenetic subgroups of B-cell precursor (BCP) ALL

	No. of patients, (%)	Median WBC $\times 10^9/L$ (75% range)	$P$ value vs. normal karyotype	Correlation between WBC and age, $r_s$
T-ALL	267	86.8 (8.8–394.5)		$-0.06$ , $P = 0.33$
Boys	197 (73.8)	97.2 (9.3–388.8)		$-0.09$ , $P = 0.23$
Girls	70 (26.2)	70.0 (4.5–435.6)		$-0.006$ , $P = 0.96$
BCP	2371	8.6 (2.3–56.5)		$-0.17$ , $P < 0.001$
Boys	1246 (52.6)	9.0 (2.5–60.9)		$-0.15$ , $P < 0.001$
Girls	1125 (47.4)	6.2 (2.2–87.5)		$-0.20$ , $P < 0.001$
Normal karyotype	445 (21.3)	7.6 (2.2–43.9)		$-0.09$ , $P = 0.05$
HeH	664 (31.8)	6.7 (2.2–32.1)	0.16	$-0.28$ , $P < 0.001$
t(12;21)	375 (18.0)	10.4 (2.9–60.7)	0.003	$-0.27$ , $P < 0.001$
t(1;19)	47 (2.2)	17.0 (6.2–72.0)	$< 0.001$	$-0.04$ , $P = 0.80$
dic(9;20)	31 (1.5)	22.0 (5.0–93.7)	$< 0.001$	$-0.24$ , $P = 0.20$
amp(21)	14 (0.7)	6.6 (1.7–24.0)	0.37	$-0.30$ , $P = 0.30$
11q23/ <i>MLL</i>	29 (1.4)	52.5 (3.6–475.5)	$< 0.001$	0.41, $P = 0.03$
t(9;22)[ <i>BCR/ABL</i> ]	41 (2.0)	29.6 (2.6–229.8)	$< 0.001$	$-0.11$ , $P = 0.48$
Hypodiploid	19 (0.9)	12.3 (3.3–100.4)	0.17	$-0.26$ , $P = 0.28$
Other	345 (16.5)	11.4 (2.8–67.0)	$< 0.001$	$-0.13$ , $P = 0.02$
Down syndrome	54 (2.1)	16.1 (2.2–66.6)	0.007	$-0.04$ , $P = 0.77$

WBC = white blood cell count in peripheral blood at diagnosis;  $P$  value = determined after comparison by Mann–Whitney  $U$ -test of the white blood cell count in the cytogenetic subgroups vs. normal karyotype; high-hyperdiploid karyotype = modal chromosome number  $> 50$ ; hypodiploid karyotype = modal chromosome number  $< 45$ ; other = including hypodiploid, pseudodiploid, near triploid and near tetraploid cases.  $P$ -values without Bonferroni correction.



CNS disease ( $P < 0.001$ ), both for the 27 T-lineage cases (10.2% of all the T-ALL patients; median WBC: 164.0 vs.  $73.0 \times 10^9/L$  for CNS positive and negative, respectively,  $P = 0.03$ ) and for the 40 BCP cases (1.7% of all BCP patients; median WBC: 10.8 vs.  $8.6 \times 10^9/L$  for CNS positive and negative, respectively,  $P = 0.01$ ). For patients with a WBC  $\geq 100.0 \times 10^9/L$ , the frequency of CNS involvement was more than twice as high as for those with WBC  $< 100.0 \times 10^9/L$  for T-ALL (16.6% vs. 6.4%,  $P = 0.03$ ), but not for BCP ALL (3.5% vs. 1.6%,  $P = 0.20$ ). The median WBC was also moderately higher for the 15 patients with overt testicular leukaemia at diagnosis (1.1% of the 1425 evaluable male patients) (median WBC: 25.1 vs.  $11.0 \times 10^9/L$ , respectively,  $P = 0.03$ ) and that was the case for both the six T-ALL (median WBC: 137.0 vs.  $97.0 \times 10^9/L$ ) and the nine BCP cases (median WBC: 21.6 vs.  $8.9 \times 10^9/L$ ). Among the 127 T-ALL patients with a WBC  $\geq 100.0 \times 10^9/L$  at diagnosis, those 19 patients with extramedullary disease (CNS and/or testis positive) did significantly worse than the remaining 108 patients (pEFS<sub>10</sub> 0.37 and 0.63, respectively,  $P = 0.004$ ). For the BCP patients, the low number of patients did not allow for such comparisons.

### Response to therapy and event-free survival

The 2048 patients who stayed in first remission had a median follow-up of 8.5 yr (75% range 2.7–14.5 y.). In total, 36 patients have died during induction therapy (1.4%), 22 had resistant disease (0.8%), 48 patients have died in CR1 (1.8%), 26 patients (1.0%) have developed a second malignant neoplasm, and 458 patients (17.4%) had experienced a leukaemic relapse at a median of

2.5 yr from diagnosis (75% range: 9–53 months). The 10-yr event-free (pEFS<sub>10y</sub>) survival and overall survival (pOS<sub>10y</sub>) were  $0.75 \pm 0.01$  and  $0.85 \pm 0.01$ , respectively. The remission rate was higher for BCP ALL (98.4%) than for T-ALL (92.5%) ( $P < 0.001$ ). The patients who achieved remission had lower WBC at presentation than patients with resistant disease or induction failure for BCP ALL (median WBC: 8.3 vs.  $24.8 \times 10^9/L$ ,  $P = 0.001$ ), but that was not the case for T-ALL (median WBC: 86.8 vs.  $127.8 \times 10^9/L$ ,  $P = 0.5$ ).

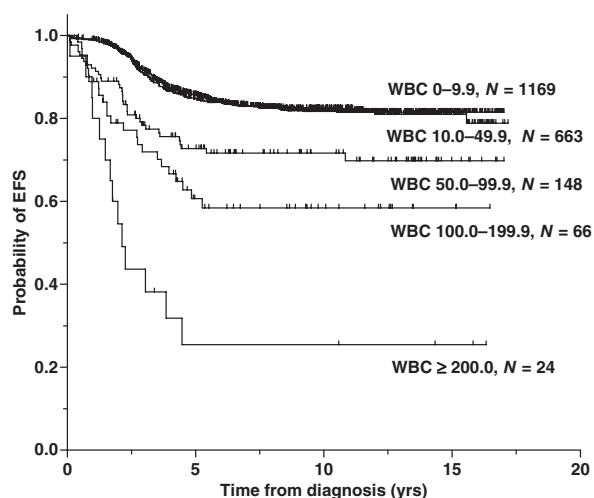
In spite of treatment stratification according to WBC at diagnosis, univariate analysis revealed a significant impact of WBC on the pEFS for BCP ALL (Table 2). The risk for an event was correlated with higher WBC for BCP even after exclusion of Down syndrome and cases bearing other risk factors that were used for treatment stratification in NOPHO 92 and 2000 protocols, ( $P < 0.001$ ) (Fig. 1). Furthermore, there was a trend towards more events for the patients with higher WBC within some of the relatively homogeneous cytogenetic BCP subgroups (Table 2), but this reached significance only for the patients with HeH ( $P = 0.04$ ) or a normal karyotype ( $P < 0.001$ ) ( $P$ -values after Bonferroni correction). In contrast, there was no significant difference in pEFS between subgroups defined by WBC at diagnosis for T-lineage ALL, but their overall EFS was relatively low (Table 2).

To explore the impact of WBC and the early response to therapy, defined by MRD<sub>d29</sub>, on the outcome, we subdivided patients into the groups of WBC  $< 100.0$  vs. WBC  $\geq 100.0 \times 10^9/L$  at diagnosis, which is a risk stratifying factor in the NOPHO ALL 2008 protocol. The pEFS for the patients who had MRD<sub>d29</sub>  $< 10^{-3}$  was excellent irrespective of a WBC  $< 100.0$  or WBC  $\geq 100.0$

**Table 2** Log rank test for the probability of 10-yr event-free survival (pEFS<sub>10y</sub>) for cytogenetic subgroups of B-cell precursor (BCP) and T-lineage ALL (T-ALL) patients stratified by white blood cell count at diagnosis

WBC, $\times 10^9/L$	pEFS <sub>10y</sub> (N)				P value
	<10.0	10–49.9	50–99.9	>100.0	
BCP	0.80 (1241)	0.80 (748)	0.70 (189)	0.49 (139)	<0.001
HeH	0.82 (410)	0.81 (205)	0.68 (35)	0.48 (13)	0.004
t(12;21)	0.77 (180)	0.82 (130)	0.83 (33)	0.70 (21)	0.41
t(1;19)	0.80 (10)	0.84 (28)	0.80 (5)	–	0.64
dic(9;20)	0.78 (9)	0.64 (9)	0.63 (8)	0.67 (3)	0.53
amp(21)	0.44 (9)	0.50 (4)	–	–	0.52
11q23/MLL	0.88 (8)	0.80 (5)	0.50 (6)	0.31 (10)	0.009
t(9;22)[BCR/ABL]	0.53 (11)	0.39 (13)	0.80 (5)	0.17 (12)	0.19
Hypodiploid	0.50 (8)	0.50 (8)	–	0.67 (3)	0.94
Normal karyotype	0.82 (248)	0.83 (132)	0.71 (25)	0.46 (22)	<0.001
T-ALL	0.58 (38)	0.70 (64)	0.65 (38)	0.58 (127)	0.33
Down	0.59 (20)	0.58 (26)	0.40 (5)	0.33 (3)	0.05

WBC = white blood cell count in peripheral blood at diagnosis; N = number of patients; pEFS<sub>10y</sub> = probability of 10-yr event-free survival; HeH = high-hyperdiploid karyotype (modal chromosome number  $>50$ ); hypodiploid karyotype = modal chromosome number  $<45$ ; 11q23/MLL = 11q23/MLL rearrangement; Ph+ = translocation t(9;22)[BCR/ABL].  $P$ -values without Bonferroni correction.



**Figure 1** Probability of event-free survival (pEFS) for B-cell precursor (BCP) ALL by white blood cell count (WBC) in peripheral blood at diagnosis [excluding patients with CNS or testis involvement, mediastinal mass, lymphomatous disease, translocations  $t(9;22)[BCR/ABL]$  or  $t(1;19)(q23;p13)$ ,  $11q23/MLL$  aberration or a hypodiploid karyotype ( $<45$  chromosomes) and patients with Down syndrome].

$\times 10^9/L$  at diagnosis both for BCP ALL (pEFS<sub>5y</sub> 0.86 and 0.85) and for T-ALL (pEFS<sub>5y</sub> 0.86 and 0.85) (Fig. 2A). In contrast, for those with MRD<sub>d29</sub>  $\geq 10^{-3}$ , but in morphological remission at day 29 ( $<5\%$  leukaemic blasts in bone marrow), the patients with WBC  $\geq 100.0 \times 10^9/L$  had an inferior pEFS compared to the patients with lower WBC (pEFS<sub>5y</sub> 0.50 and 0.75,  $P = 0.001$ ) (Fig. 2B) and that was the case both for BCP [pEFS<sub>5y</sub> 0.58 ( $N = 11$ ) vs. 0.76 ( $N = 145$ )] and for T-ALL [pEFS<sub>5y</sub> 0.38 ( $N = 8$ ) vs. 0.71 ( $N = 7$ )].

The prognostic impact of WBC on the risk of event was explored further by a multivariate Cox regression analysis including gender, age, WBC, MRD<sub>d29</sub> and cytogenetic aberrations for BCP (grouped as: (i) normal karyotype, (ii) favourable cytogenetics [defined as HeH or translocation  $t(12;21)$ ], (iii) unfavourable cytogenetics [defined as hypodiploid karyotype or  $11q23/MLL$  aberration or translocation  $t(9;22)$  or  $dic(9;20)$ ] or (iv) other (defined as the presence of all the other aberrations including cases with an aberrant, but uninformative karyotype). Age, WBC and MRD<sub>d29</sub> were analysed as continuous variables, and gender as well as cytogenetic aberrations as categorical variables. Because MRD<sub>d29</sub> data were available almost exclusively (except in four cases) for ALL-2000 protocol patients, and there were differences in treatment approaches between the two protocols, only the ALL-2000 patients were analysed. Patients for whom remission was not achieved [15 induction failures (1.5%) and 14 resistant disease (1.4%)] as well as 24 with Down syndrome (2.4%) were excluded. Of the remaining 965 cases, MRD<sub>d29</sub> data were unavail-

able for 246 patients leaving 719 patients (74.5%) available for the analysis. After stratifying the analysis by immunophenotype, the stratified Cox regression analysis showed WBC to be a significant independent risk factor (hazard ratio being 1.002,  $P = 0.006$ ). Other significant independent risk factors were MRD<sub>d29</sub> (hazard ratio 1.055,  $P < 0.001$ ), and for the BCP patients there was an increased risk for those with poor cytogenetics (hazard ratio 3.5,  $P < 0.001$ ).

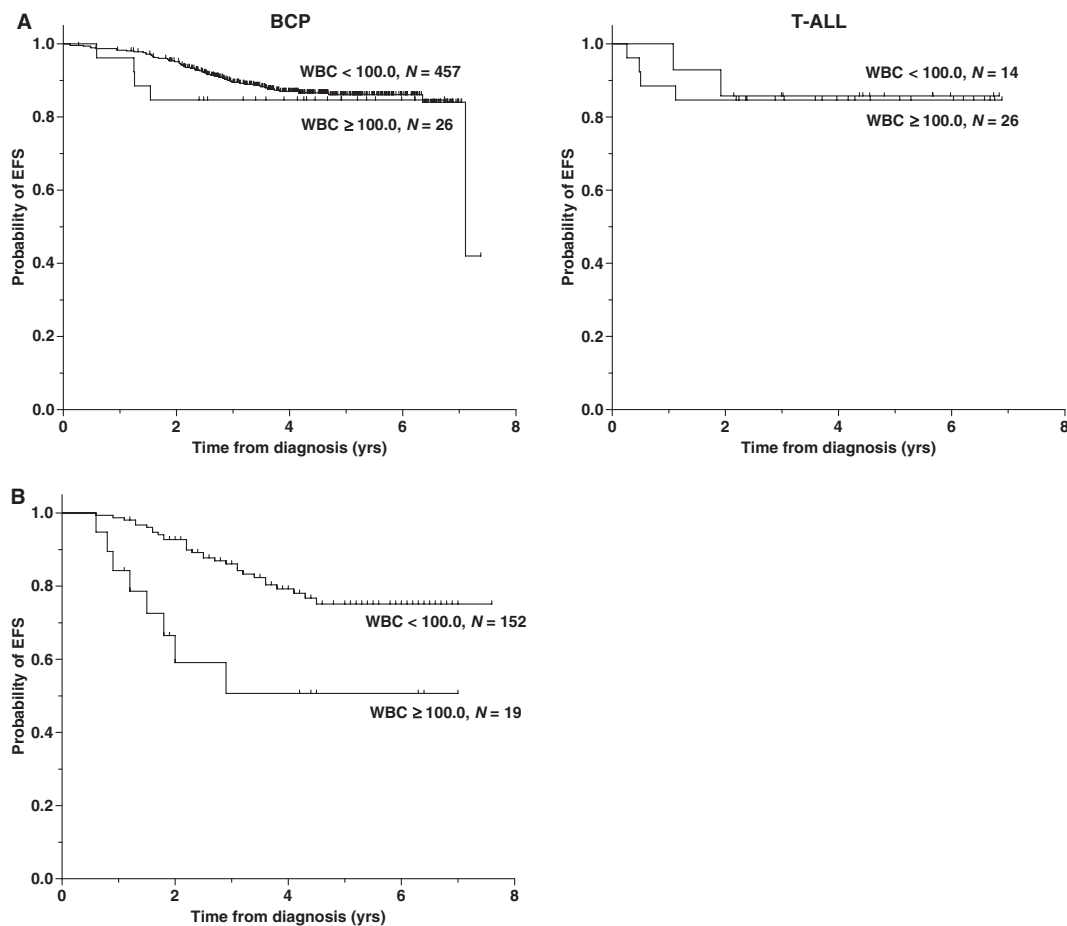
## Discussion

Relatively little is known about the biological mechanisms that control leukaemic cell proliferation and the propensity of leukaemic cells to migrate out from the bone marrow and escape from apoptosis outside the microenvironment of the bone marrow and lymphoid tissues (25–27). Thus, the WBC at diagnosis in a child with ALL can be influenced by its cellular origin (stages of B- and T-lineage differentiation), the genetic aberrations of the leukaemic clone (7, 28), the microenvironment from which it emerged and the cytokine profile in peripheral blood, which may be influenced by both age and gender (29–32). Finally, the WBC at diagnosis of ALL includes both leukaemic blasts with a limited proliferation potential and true clonogenic leukaemic stem cells (33), as well as normal leucocytes that are often present in peripheral blood and may significantly influence the total WBC, when the peripheral blast count is low.

The current study demonstrates the heterogeneity of WBC distribution among the subsets of patients with ALL defined by their clinical presentation at diagnosis and early molecular response to therapy. Furthermore, our results emphasise that the prognostic impact of WBC at diagnosis reflects leukaemia subsets rather than just tumour burden, and this is linked to the immunophenotype and karyotype of the leukaemic cells and the molecular response to therapy. Our findings have several implications.

First, 97% of ALL cases with WBC within normal range at diagnosis are of BCP origin, while for those with WBC  $\geq 100.0 \times 10^9/L$ , BCP and T-ALL occurred at almost the same frequency. Patients with higher WBC show higher propensity for extramedullary and extralymphatic presentation, which demonstrates their different abilities to evade apoptosis, to survive outside the bone marrow microenvironment and to infiltrate non-lymphatic organs. These findings question whether the presence of extramedullary disease carries an independent prognostic significance beyond that determined by the WBC.

Second, the proportion of HR BCP cytogenetic subsets increases with increasing WBC. Accordingly, the poor prognosis of the cases with high WBC is strongly associated with poor-risk karyotypes and not just with tumour



**Figure 2** Probability of event-free survival (pEFS) for the B-cell precursor (BCP) and T-ALL with minimal residual disease at day 29 (A)  $<0.1\%$  ( $\text{MRD}_{\text{d}29} < 10^{-3}$ ), with respect to white blood cell count in peripheral blood (WBC) at diagnosis,  $P = 0.61$ . (B) more than  $0.1\%$  ( $\text{MRD}_{\text{d}29} \geq 10^{-3}$ ) and in remission ( $<5\%$  leukaemic blasts in bone marrow), with respect to white blood cell count in peripheral blood (WBC) at diagnosis,  $P = 0.001$ .

burden (34). However, even among the relatively homogeneous BCP subgroups without known unfavourable cytogenetics such as those with a normal karyotype, there is an inverse relationship of WBC to event-free survival, which could support tumour burden as an independent risk factor or indicate the presence of rare cytogenetic aberrations that cause both a high WBC and a poor prognosis as it was shown within the subsets of patients with known cytogenetic aberrations as *MLL* gene rearrangement where the cases with different partner genes showed different aspects of clinical picture and outcome (35, 36).

Third, the present study confirms previous findings, demonstrating that the outcome for the patients with a rapid reduction in tumour burden to  $<10^{-3}$  at the end of induction overrides and eliminates some of the classical risk factors such as  $\text{WBC} \geq 100.0 \times 10^9/\text{L}$  and T-ALL (37). To test whether patients with high tumour burden but rapid early response can do well with less aggressive treatment, the current NOPHO ALL-2008 protocol downgrades patients with  $\text{WBC} \geq 100.0 \times 10^9/\text{L}$

at diagnosis, no *MLL* rearrangements or hypodiploidy, and low levels of MRD at the end of induction to less intensive therapy. In contrast, among the slow responders ( $\text{MRD}_{\text{d}29} \geq 10^{-3}$ ), a  $\text{WBC} \geq 100.0 \times 10^9/\text{L}$  still identifies a subset of patients with a significantly poorer prognosis even though they have received more intensive therapy. Whereas BCP patients with high WBC and a poor response to initial treatment clearly require more intensive therapy and potentially even SCT in first remission, the impact of WBC on EFS is less clear for T-ALL because of the low number of patients included into the present study.

## Conclusion

Previously, the target of risk grouping was to identify patient subsets with increased risk of treatment failure. Today, however, ALL subsets defined by genetic profiling, early MRD response or drug dispositions have cure rates of around 95% or higher. Because these are candi-

dates to significant reductions in treatment intensity or treatment duration, it is important to determine whether the subset of patients with very high WBC at diagnosis should be excluded from such very good risk groups. Finally, the factors that determine the WBC levels at diagnosis including leukaemia molecular biology and host factors remain to be determined and may in the future refine risk grouping.

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### Conflict of interest

The authors declare no competing financial interests.

### Contribution

G. Vaitkevičienė and K. Schmiegelow designed the study and wrote the paper; E. Forestier (together with the Nordic Society for Paediatric Oncology and Haematology cytogenetic group) scrutinised the karyotypes of all patients; S. Roesthoej revised statistical analysis; M. Heyman chairs the NOPHO Leukaemia registry and was together with S. Söderhäll responsible for collecting the data in Sweden; M. Hellebostad, O. G. Jonsson, P. M. Lähteenmäki and K. Schmiegelow were responsible for providing the clinical and follow-up data for patients from Norway, Iceland, Finland and Denmark, respectively. All authors commented and approved the final paper.

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### Supporting Information

Additional supporting information may be found in the online version of this article:

**Table S1.** Treatment protocols – NOPHO ALL-92.

**Table S2.** Treatment protocols – NOPHO ALL-2000.

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