High modal number and triple trisomies are highly correlated favorable factors in childhood B-cell precursor high hyperdiploid acute lymphoblastic leukemia treated according to the NOPHO ALL 1992/2000 protocols

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ABSTRACT

Between 1992 and 2008, 713 high hyperdiploid acute lymphoblastic leukemias in children aged 1-15 years were diagnosed and treated according to the Nordic Society for Pediatric Hematology and Oncology acute lymphoblastic leukemia 1992/2000 protocols. Twenty (2.8%) harbored t(1;19), t(9;22), der(11q23), or t(12;21). The median age of patients with "classic" high hyperdiploidy was lower than that of patients with translocation-positive high hyperdiploidy (P<0.001). Cases with triple trisomies (+4, +10, +17), comprising 50%, had higher modal numbers than the triple trisomy-negative cases (P<0.0001). The probabilities of event-free survival and overall survival were lower for those with white blood cell counts ≥50x10^9/L (P=0.017/P=0.009), ≥5% bone marrow blasts at day 29 (P=0.001/0.002), and for high-risk patients (P<0.001/P=0.003), whereas event-free, but not overall, survival, was higher for cases with gains of chromosomes 4 (P<0.0001), 6 (P<0.003), 17 (P=0.010), 18 (P=0.049), and 22 (P=0.040), triple trisomies (P=0.002), and modal numbers >53/55 (P=0.020/0.024). In multivariate analyses, modal number and triple trisomies were significantly associated with superior event-free survival in separate analyses with age and white blood cell counts. When including both modal numbers and triple trisomies, only low white blood cell counts were significantly associated with superior event-free survival (P=0.009). We conclude that high modal chromosome numbers and triple trisomies are highly correlated prognostic factors and that these two parameters identify the same subgroup of patients characterized by a particularly favorable outcome.

Introduction

High hyperdiploid (HeH) cases (51-67 chromosomes), characterized by non-random multiple chromosomal gains, form one of the largest subgroups of pediatric B-cell precursor acute lymphoblastic leukemia (ALL).[1] HeH cases are generally associated with favorable clinical features, such as a low white blood cell (WBC) count, lack of extramedullary manifestations, and age between 2-5 years, and have a superior outcome, with overall survival (OS) rates exceeding 90% in most contemporary treatment protocols.[1-7] This notwithstanding, a substantial proportion of patients relapses, so event-free survival (EFS) rates are only 70-80%.[4-7] Several attempts have been made to define the clinical and genetic characteristics present at the time of diagnosis that would enable identification of patients who will respond poorly to standard-risk treatment and who could, therefore, benefit from more intensive therapy. However, only a few clinical features have been shown to be associated with decreased EFS within the HeH group, namely age >10 years, male gender, and bone marrow fibrosis.[5,8,9] As regards genetic factors, it has been suggested that some karyotypic patterns and aberrations – low modal chromosome number, lack of certain trisomies, and the presence of various structural changes – confer a more dismal outcome, albeit with conflicting results in different studies.[2,4,5,7,9-13] The prognostic impact of the "triple trisomies", i.e., concur-

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rent +4, +10, and +17, is debated; they have been reported to be associated with low risk by the Children's Oncology Group (COG) but not in UK trials.[7,13] Furthermore, +18 has been correlated with a lower risk of relapse, at least in the UK.[5,7] In order to address the important issue of clinical and genetic risk factors in HeH, we ascertained and reviewed all such cases diagnosed between 1992 and 2008 in Nordic countries and treated according to the Nordic Society for Pediatric Hematology and Oncology (NOPHO) ALL 1992 and ALL 2000 protocols,[14] a cohort of more than 700 patients.

Methods

Patients and cytogenetics

The following inclusion criteria were applied: B-cell precursor ALL diagnosed in children aged 1-15 years in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) between 1992 and 2008 that were high hyperdiploid (51-67 chromosomes) or, in cases in which cytogenetic analysis failed or those with a "normal" karyotype, showing a gain of more than four extra chromosomes by interphase fluorescence *in situ* hybridization (FISH) or having a DNA index greater than 1.12.[1,15,16] Chromosome banding analyses were performed on bone marrow/peripheral blood samples using standard methods by 15 different cytogenetic laboratories in the Nordic countries. All abnormal karyotypes have been centrally reviewed annually since 1996 in Sweden and since 2000 in all five Nordic countries. Cases with t(1;19)(q23;p13)/*TCF3-PBX1*, t(9;22)(q34;q11)/*BCR-ABL1*, der(11q23)/*MLL* rearrangement, or t(12;21)(p13;q22)/*ETV6-RUNX1* in a high hyperdiploid background (translocation-positive high hyperdiploidy; t-HeH) were not excluded from the initial analyses, nor were cases with incomplete karyotypes. Since 2000, the NOPHO protocols[14] have required, apart from conventional G-banding, targeted analyses for t(1;19), t(9;22), der(11q23), and t(12;21) by FISH, Southern blot, or reverse transcriptase-polymerase chain reaction (PCR). Although such investigations were not compulsory in the ALL 1992 protocol,[14] several of the participating laboratories had performed prospective analyses of the above-mentioned rearrangements since the mid 1990s, in some instances also retrospectively.

The study was approved by the Regional Ethical Review Board at Lund University and informed consent was obtained according to the Declaration of Helsinki.

Statistical analyses

The PASW Statistics 18.0.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used for all calculations. The significance limit for two-sided *P* values was set at less than 0.05. Modal chromosome numbers were defined as the highest number in karyotypes with a range of chromosome numbers. In the analysis of the prognostic impact of +4, +10, +17 (triple trisomies), and +18, cases were considered completely informative if no marker chromosomes were present; if markers were found, cases could not be classified as negative for these specific trisomies. Possible differences between subgroups were investigated using two-sided Mann-Whitney's tests, two-tailed Fisher's exact probability tests, and χ^2 tests. The probabilities of EFS (pEFS) and OS (pOS) at 5 and 10 years were calculated using the Kaplan-Meier method, and subgroups were compared using the log rank test. The following parameters were investigated: t-HeH, modal number, triple trisomies, all individual gains, structural changes as such, 1q gain, 6q deletion, 17q gain, gender, age, WBC count, mediastinal mass, central nervous system involvement, bone marrow remission at days 15 and 29, treatment protocol, and risk group (standard risk, intermediate risk, high risk).[14] Multivariate analysis using a Cox regression model was performed to identify factors that had an independent impact on pEFS and pOS. In the analysis of pEFS, events consisted of induction failure, resistant disease, relapse, death in first complete remission, and second malignant neoplasms. In the analysis of pOS, death from any cause was the endpoint. The median observation time for patients in continuous first complete remission was 126 months (range, 24-235 months). The NOPHO leukemia registry is updated annually; follow-up data were extracted in April, 2012.

Results

Basic cytogenetic features

A total of 713 cases were ascertained and reviewed, accounting for 30% of all Nordic B-cell precursor ALL in children aged 1-15 years diagnosed between 1992 and 2008. Of these, 695 (97%) displayed G-banded karyotypes with 51-67 chromosomes, ten (1.4%) were identified by interphase FISH, and eight (1.1%) by DNA flow analyses. Twenty (2.8%) cases harbored one of the ALL-associated translocations t(1;19) (n = 4), t(9;22) (n = 6), der(11q23) (n = 3), or t(12;21) (n = 7) and were classified as t-HeH. The remaining 693 were "classic" HeH B-cell precursor ALL.

Cytogenetic features of the 693 high hyperdiploid cases

Of the 693 cases, modal chromosome numbers were known in 675; the median mode was 55 chromosomes (*Online Supplementary Figure S1A*). Triple trisomies (+4,+10,+17) were present in 175 (50%) and absent in 178 (50%) of 353 informative cases. The triple trisomy-positive cases had a median mode of 56 chromosomes (range, 51-65), whereas the negative cases had a median mode of 55 chromosomes (range, 51-62) (*Online Supplementary Figure S1B and C*) ($P<0.0001$).

A total of 298 cases were informative for all chromosomes as regards numerical changes. The most frequently gained chromosomes were 21 (98.4%), X (90.0%), 6 (83.2%), 14 (82.8%), 18 (77.7%), 4 (77.3%), 17 (73.1%), 10 (71.2%), and 8 (37.5%) (*Online Supplementary Figure S2*). All other chromosomes were gained in less than 25% of cases.

Among 456 informative cases, 237 (52%) had structural chromosome changes, with the most common being 1q gain (68/337; 20%), 6q deletion (14/318; 4.4%), and 17q gain (11/311; 3.5%).

Cytogenetic features of the 20 translocation-positive high hyperdiploid cases

The karyotypes of the t-HeH cases are listed in *Online Supplementary Table S1*. The median modal chromosome numbers were 57 for the t(1;19) group, 56-57 for t(9;22), 54 for der(11q23), and 52 for t(12;21). Among the nine informative cases, the most frequent gains were +X (89%), +21 (89%), +6 (67%), +10 (67%), +4 (56%), +14 (56%), and +18 (44%). All other chromosomes were gained in less than 25% of cases.

Clinical features at diagnosis

The male/female sex ratio was close to 1.0 in all cytogenetic subgroups, with the possible exception of t(1;19)-positive cases (Table 1). Two patients had Down syndrome (DS), neither of whom had t-HeH. The median age

was lower (P<0.001) among the HeH cases (3.78 years; range, 0.95-15.0) than the t-HeH cases (5.92 years; range, 2.86-14.19), whereas no significant differences were seen between these groups regarding WBC counts or incidence of extramedullary leukemia.

Clinical features at diagnosis in relation to karyotypic patterns of the high hyperdiploid cases

Among the 688 HeH cases treated according to the NOPHO ALL 1992/2000 protocols,[14] 670 were informative for modal chromosome numbers. Clinical features and survival in relation to modal number are given in *Online Supplementary Table S2*. There were no significant differences in age or WBC count distributions between cases with 51-53 chromosomes *versus* cases with more than 53 chromosomes or between cases with 51-55 chromosomes *versus* cases with more than 55 chromosomes. The modal chromosome number distributions did not differ between boys and girls.

As regards cases positive and negative for triple trisomies, there was no impact of age, WBC count, or gender. Among the most frequent gains, there were significant differences in age distributions for chromosomes 6 (median age 4.74 years for disomy *versus* 3.42 for trisomy; P<0.001), 17 (median 4.65 for disomy *versus* 3.43 for trisomy; P=0.001), and 18 (median age 4.51 years for disomy *versus* 3.44 for trisomy; P=0.021) and in WBC counts for chromosome 4 (median WBC count 10.8 for disomy *versus* 6.1 for trisomy; P=0.036). Gains of chromosomes 4 and 6 were more common in females than in males (85% *versus* 71%; P=0.002 and 89% *versus* 80%; P=0.021). The presence of structural changes as such, 1q gain, 6q deletion, or 17q gain did not vary in relation to WBC counts or gender, whereas the age distribution differed significantly between cases with and without structural changes (median age 4.10 *versus* 3.76; P=0.028).

Risk classification and treatment response

Data on risk stratification and treatment response, including frequency and types of primary events, in the 688 HeH and the 20 t-HeH patients treated according to the NOPHO ALL 1992/2000 protocols, are summarized in Table 2. Since there were no significant differences in pEFS at 5 and 10 years between patients treated according to the ALL 1992 or 2000 protocols [ALL 1992: 0.82, standard error (SE) 0.02 and 0.79 SE 0.02, respectively, *versus* ALL 2000: 0.82 SE 0.02 and 0.81 SE 0.02, respectively; P=0.683] or in pOS at 5 and 10 years (ALL 1992: 0.90 SE 0.02 and 0.88 SE 0.02, respectively, *versus* ALL 2000: 0.92 SE 0.02 and 0.91 SE 0.02, respectively; P=0.297), data from these two treatment regimens were combined in the subsequent analyses.

Forty-nine percent of the HeH cases were classified as standard risk, 38% as intermediate risk, and 14% as high risk (Table 2). The corresponding frequencies for t-HeH were 40%, 5%, and 55%; however, it should be stressed that the presence of t(1;19), t(9;22), and der(11q23) was risk-stratifying in the protocols. The majority of patients, irrespectively of whether they had HeH or t-HeH, were in morphological remission at day 15 and close to 100% had less than 5% bone marrow blasts at day 29 (Table 2). There were no significant differences between the HeH and t-HeH groups as regards achievement of complete remission or in proportions of patients in first complete remission, induction failure/resistant disease, relapse, death in first complete remission, or development of a second malignant neoplasm.

Survival in relation to clinical features among the 708 patients treated according to the NOPHO ALL 1992/2000 protocols

For the entire patient cohort, the 5- and 10-year pEFS were 0.82 and 0.80, respectively; the corresponding pOS were 0.91 and 0.89 (*Online Supplementary Table S3*; *Online Supplementary Figure S3*). There were no significant differences in pEFS (P=0.439) or pOS (P=0.393) between the 688 HeH and 20 t-HeH patients.

The impact of gender, age, WBC count, central nervous system disease, mediastinal mass, risk group, and percentage of bone marrow blasts at days 15 and 29 on pEFS and pOS was investigated in the 688 HeH patients. Survival rates were significantly lower for boys (pEFS only; P=0.006; Figure 1), for patients with WBC counts ≥50x10^9/L (pEFS; P=0.017 and pOS; P=0.009; Figure 2) or ≥5% bone marrow blasts at days 15 (pEFS; P<0.001 and pOS; P<0.001) and 29 (pEFS; P=0.001 and pOS; P=0.002), and for patients grouped as high risk (pEFS; P<0.001 and pOS; P=0.003) (Table 3).

Survival in relation to cytogenetic features among the 688 high hyperdiploid patients treated according to the NOPHO ALL 1992/2000 protocols

The presence of trisomies/tetrasomies 4, 6, 17, 18, and 22 was significantly associated with a higher pEFS; none

Table 1. Clinical features at the time of diagnosis of 713 children with high hyperdiploid B-cell precursor ALL.

	Total (n = 713; 100%)	HeH (n = 693; 97.2%)	t(1;19)[1] (n = 4; 0.6%)	t(9;22)[1] (n = 6; 0.8%)	der(11q23)[1] (n = 3; 0.4%)	t(12;21)[1] (n = 7; 1.0%)
Male/female (ratio)	359/354 (1.0)	347/346 (1.0)	3/1 (3.0)	3/3 (1.0)	2/1 (2.0)	4/3 (1.3)
Down syndrome (%)[2]	2 (0.3%)	2 (0.3)	0	0	0	0
Median age in years (range)	3.85 (0.95-15.0)	3.78 (0.95-15.0)	6.58 (5.23-7.45)	11.6 (5.37-14.2)	3.85 (3.72-4.01)	4.68 (2.86-13.2)
Median WBC x 10^9/L (range)	6.5 (0.4-291)	6.5 (0.4-291)	2.4 (2.1-7.0)	62.8 (2.0-106)	10.6 (4.1-30.7)	4.0 (1.5-10)
Extramedullary leukemia (%)[2]						
Central nervous system	13/706 (1.8)	11/686 (1.6)	0/4	1/6 (17)	1/3 (33)	0/7
Mediastinal mass	9/706 (1.3)	9/686 (1.3)	0/4	0/6	0/3	0/7
Testes[3]	3/354 (0.8)	3/342 (0.9)	0/3	0/3	0/2	0/4

[1]In cases with 51-67 chromosomes; [2]data missing for some variables in the HeH cases: Down syndrome (n=8), central nervous system involvement (n=7), mediastinal mass (n=7), and testicular involvement (n=5); [3]percentage of males. HeH: high hyperdiploidy without t(1;19)(q21;q23), t(9;22)(q34;q11), der(11q23), or t(12;21)(p13;q22); WBC: white blood cell count.

of the gains correlated with pOS (*Online Supplementary Table S4*). Cases positive for triple trisomies (P=0.003) or with modal numbers >53/55 (P=0.020/0.031) had higher pEFS; there was no significant difference in pOS. Neither pEFS nor pOS varied significantly with the presence of structural changes as such, 1q gain, 6q deletion, or 17q gain.

Multivariate analysis of clinical and cytogenetic features in relation to survival among the high hyperdiploid patients

A total of 344 HeH patients treated according to the NOPHO ALL 1992/2000 protocols were completely informative for age, WBC count, triple trisomies, and modal number. Compared with the HeH cases lacking data on modal chromosome number and/or triple trisomies, this subgroup of 344 patients did not differ significantly as regards pOS (P=0.335) whereas pEFS was somewhat increased (P=0.041).

A multivariate analysis of age, WBC count, and modal numbers as continuous variables revealed that all these three parameters were significantly associated with pEFS, with high modal numbers (P=0.003; Figure 3), low WBC counts (P=0.009), and young age (P=0.027) being associated with favorable outcome, whereas a multivariate analysis of age, WBC count, and triple trisomies showed that low WBC counts (P=0.011) and presence of triple trisomies (P=0.004; Figure 4) were associated with superior pEFS; in this analysis, young age was not significant (P=0.09). When including both modal numbers and triple trisomies, only low WBC counts were significantly associated with superior pEFS (P=0.009); the three other parameters were not significant – young age (P=0.052), high modal numbers (P=0.056), and presence of triple trisomies (P=0.081).

As regards pOS, a multivariate analysis of age, WBC count, and modal numbers as continuous variables revealed that only young age was significantly associated with superior survival (P=0.021), whereas low WBC counts and high modal numbers were not (P=0.069 and P=0.1, respectively). The same was true when including triple trisomies instead of modal number (young age: P=0.045; low WBC counts: P=0.084; presence of triple trisomies: P=0.1) and when including both modal numbers and triple trisomies (young age: P=0.034; low WBC counts: P=0.079; presence of triple trisomies: P=0.33; high modal number: P=0.30).

Discussion

The present population-based, consecutive, and uniformly treated series of HeH childhood ALL, comprising 30% of all B-cell precursor ALL cases diagnosed in the

Table 2. Risk classification, response, and primary events in the 708 children with high hyperdiploid B-cell precursor ALL treated according to the NOPHO ALL 1992/2000 protocols.

	Total (n = 708; 100%)	HeH (n = 688; 97.2%)	t(1;19)[1] (n = 4; 0.6%)	t(9;22)[1] (n = 6; 0.8%)	der(11q23)[1] (n = 3; 0.4%)	t(12;21)[1] (n = 7; 1.0%)
Treatment protocol (%)						
ALL 1992	389 (55)	378 (55)	2 (50)	6 (100)	2 (67)	1 (14)
ALL 2000	319 (45)	310 (45)	2 (50)	0[3]	1 (33)	6 (86)
Classification (%)						
Standard risk	344 (49)	336 (49)	3 (75)	0	1 (33)	4 (57)
Intermediate risk	260 (37)	259 (38)	0	0	0	1 (14)
High risk	104 (15)	93 (14)	1 (25)	6 (100)	2 (67)	2 (29)
BM blasts day 15/day 29 (%)[2]						
<5%	444 (77)/628 (96)	434 (77)/609 (96)	2 (100)/4 (100)	3 (75)/6 (100)	2 (100)/3 (100)	3 (50)/6 (86)
5-25%	102 (18)/23 (3.5)	101 (18)/22 (3.5)	0/0	0/0	0/0	1 (17)/1 (14)
>25%	30 (5.2)/3 (0.5)	27 (4.8)/3 (0.5)	0/0	1 (25)/0	0/0	2 (33)/0
Complete remission (%)[2]	688 (98.7)	669 (98.7)	3 (100)	6 (100)	3 (100)	7 (100)
Primary events (%)						
Fist complete remission	572 (81)	555 (81)	4 (100)	4 (67)	2 (67)	7 (100)
Induction failure/resistant disease	8 (1.1)	8 (1.2)	0	0	0	0
Relapse	100 (14)	99 (14)	0	1 (17)	0	0
BM	72 (72)	71 (72)	0	1 (100)	0	0
CNS	11 (11)	11 (11)	0	0	0	0
Testes	1 (1.0)	1 (1.0)	0	0	0	0
BM and CNS	8 (8.0)	8 (8.1)	0	0	0	0
BM and testes	3 (3.0)	3 (3.0)	0	0	0	0
BM, CNS, and testes	1 (1.0)	1 (1.0)	0	0	0	0
BM and other site	4 (4.0)	4 (4.0)	0	0	0	0
Dead in first complete remission	17 (2.4)	16 (2.3)	0	0	1 (33)	0
Second malignant neoplasm	11 (1.6)	10 (1.5)	0	1 (17)	0	0
Acute myeloid leukemia	3 (27)	3 (30)	0	0	0	0
Myelodysplastic syndrome	6 (55)	6 (60)	0	0	0	0
Other	2 (18)	1 (10)	0	1 (100)	0	0

[1]In cases with 51-67 chromosomes; [2]data missing for some variables: BM blasts on day 15 (n=134), BM blasts on day 29 (n=55), and achievement of complete remission (n=12); [3]cases with t(9;22) were not included in the NOPHO ALL 2000 protocol. BM: bone marrow; CNS: central nervous system; HeH: high hyperdiploidy without t(1;19)(q21;q23), t(9;22)(q34;q11), der(11q23), or t(12;21)(p13;q22);

Nordic countries between 1992 and 2008, is one of the largest reported to date. The patients were characterized by young age, low WBC counts, rare occurrence of extramedullary leukemia, and an equal sex ratio, all in line with findings in previously published series.[1] Furthermore, the distribution of the modal number (*Online Supplementary Figure S1A*) and the frequencies of specific numerical (*Online Supplementary Figure S2*) and structural chromosome aberrations also agree well with other studies.[1,2,4,5] Thus, this cohort of patients is clearly representative of the HeH subgroup.

Among the 713 HeH cases, 20 (2.8%) harbored the well-known ALL-associated translocations/fusion genes t(1;19)/*TCF3-PBX1*, t(9;22)/*BCR-ABL1*, t(12;21)/*ETV6-RUNX1*, or der(11q23)/*MLL* rearrangements (*Online Supplementary Table S1*), a frequency on a par with the previously reported 1-4% t-HeH in pediatric HeH ALL.[5,17] Such cases are usually risk-grouped to the respective translocation-positive subgroup,[5] and they were, therefore, excluded from the main analyses of clinical and survival data in the present study. However, little is known about the prognostic implications of these rearrangements in an HeH context. We found no significant differences between the HeH and t-HeH groups regarding WBC counts, incidence of extramedullary leukemia, achievement of complete remission, pEFS, or pOS (Tables 1 and 2; *Online Supplementary Table S3*). On the other hand, the t-HeH patients were significantly older (Table 1). Since increased age is an established risk factor in childhood ALL,[18] this could indicate that the limited number of t-HeH cases in the present investigation precluded detection of differences in survival compared with HeH cases. However, it has been reported that t(1;19)-positive t-HeH cases are associated with favorable presenting characteristics, such as young age, low WBC counts, and lack of extramedullary leukemia, and a low incidence of

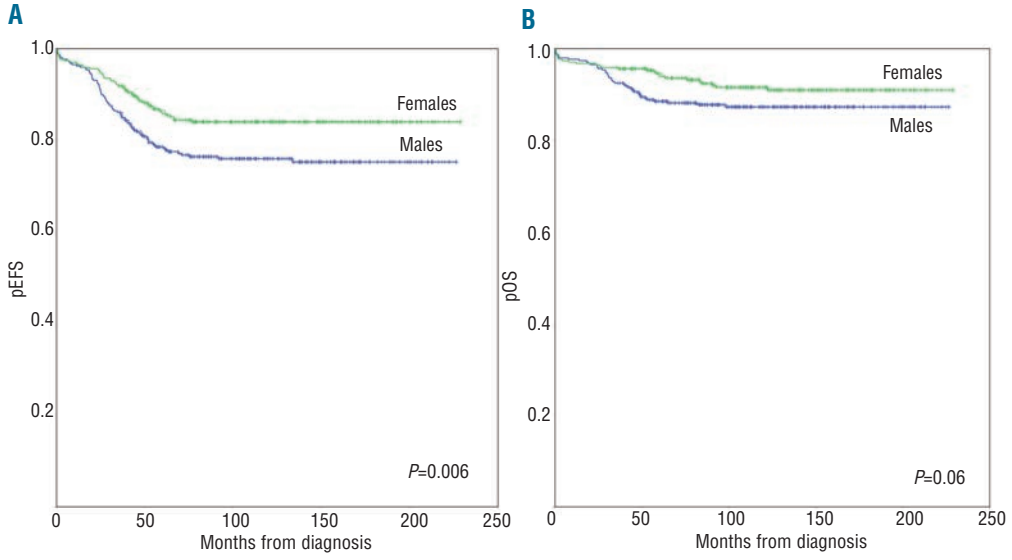

Figure 1. Event-free (A) and overall survival (B) of 688 HeH cases by gender.

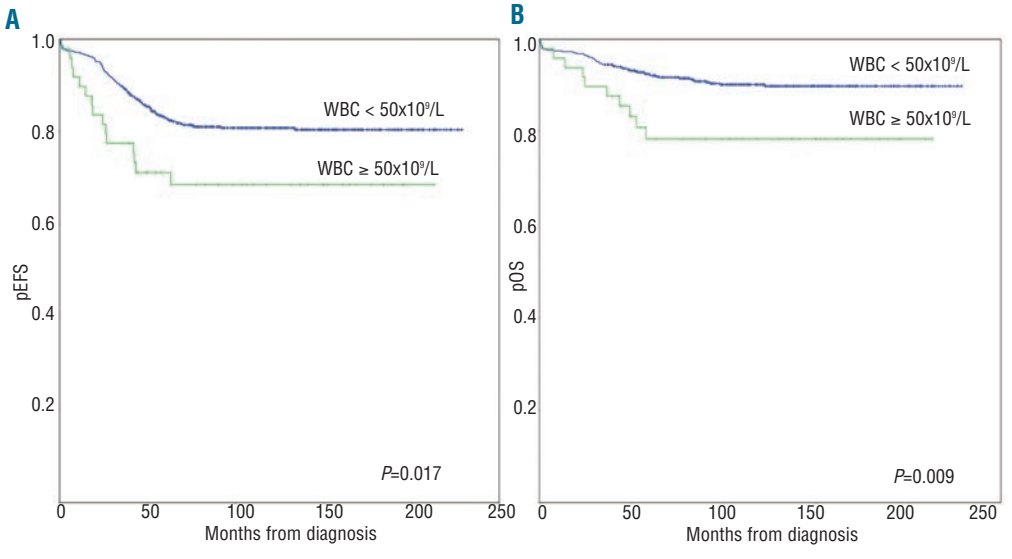

Figure 2. Event-free (A) and overall survival (B) of 688 HeH cases by white blood cell count (WBC).

relapse,[2,19] like the t(1;19)-positive cases in the present series (Tables 1 and 2; *Online Supplementary Table S3*). As far as concerns t-HeH patients with t(9;22), it has been suggested that they either have a more favorable survival and/or treatment response compared with t(9;22)-positive near-diploid ALL[20,21] or the same dismal outcome as all other patients with this translocation.[22,23] The fact that all six t-HeH with t(9;22) in our Nordic cohort achieved complete remission and all but one remain alive would rather agree with the former view (Table 2; *Online Supplementary Table S1*). Obviously, larger series are needed to address this clinically important issue, and this is something that will require international collaboration due to the rarity of t-HeH in pediatric patients.

In childhood ALL in general, several clinical features have been associated with outcome.[18] In the present HeH cohort, gender only had an impact on pEFS, whereas WBC counts, risk group, and percentage of bone marrow blasts at days 15 and 29 were significant factors for both pEFS and pOS (Table 3). Since the WBC count is an important parameter for risk stratification in the NOPHO ALL protocols,[14] it is not surprising that the risk group assignments correlated with survival considering the strong impact of high WBC counts on pEFS and pOS. That boys with HeH have a lower pEFS is in line with what was found in a previous study of a HeH series of similar size from the UK (Figure 1).[5] However, in contrast to the UK series, we also detected significantly lower pOS in cases with high WBC counts (Figure 2). These differences between the UK and the Nordic countries may well be spurious, but could also reflect different treatment regimens.

Another factor that has been associated with an unfavorable outcome in pediatric ALL is DS.[24,25] In the present study, only two (0.3%) of the patients had DS, corresponding to 2.6% of all DS-ALL diagnosed during this time period (*data not shown*). The former frequency is similar to the one described by Heerema *et al.*,[4] whereas the latter is lower than the 5-16% previously reported in DS-ALL.[26-30] Both DS patients in our series are alive in first complete remission more than 9 years after diagnosis. Thus, our data neither support a notable prevalence of DS in HeH patients nor indicate a particularly poor outcome of DS-HeH cases. However, the prognosis of DS-HeH patients must be ascertained in much larger series.

In the present study, some cytogenetic aberrations were found to be significantly associated with certain clinical features at diagnosis. For example, +4 and +6 were more common in girls than in boys. We know of no prior study reporting such gender-related cytogenetic differences in HeH, and when reviewing all published HeH karyotypes,[17] no skewed sex ratios were detected (*data not shown*). Thus, the observed gender variations may well be

Table 3. Survival in relation to clinical features of the 688 HeH patients treated according the ALL 1992/2000 protocols.

Variable[1]	N. of cases (%)	pEFS at 5 years (SE)	pEFS at 10 years (SE)	P[2]	pOS at 5 years (SE)	pOS at 10 years (SE)	P[2]
Gender							
Female	342 (50)	0.86 (0.02)	0.84 (0.02)	**0.006**	0.94 (0.01)	0.92 (0.02)	0.061
Male	346 (50)	0.79 (0.02)	0.76 (0.02)		0.89 (0.02)	0.87 (0.02)	
Age							
1-4 years	477 (69)	0.85 (0.02)	0.82 (0.02)	0.117	0.92 (0.01)	0.90 (0.01)	0.120
5-9 years	148 (22)	0.76 (0.04)	0.74 (0.04)		0.91 (0.02)	0.90 (0.03)	
10+ years	63 (9.2)	0.79 (0.05)	0.79 (0.05)		0.88 (0.04)	0.82 (0.05)	
WBC count							
<50x10^9/L	639 (93)	0.83 (0.02)	0.81 (0.02)	**0.017**	0.92 (0.01)	0.90 (0.01)	**0.009**
≥50x10^9/L	49 (7.1)	0.71 (0.07)	0.71 (0.07)		0.78 (0.06)	0.78 (0.06)	
CNS disease							
Yes	11 (1.6)	0.73 (0.13)	0.73 (0.13)	0.420	0.91 (0.09)	0.91 (0.09)	0.990
No	670 (98)	0.82 (0.02)	0.80 (0.02)		0.91 (0.01)	0.89 (0.01)	
Mediastinal mass							
Yes	9 (1.3)	0.67 (0.16)	0.67 (0.16)	0.190	0.78 (0.14)	0.78 (0.14)	0.205
No	672 (99)	0.83 (0.02)	0.80 (0.02)		0.92 (0.01)	0.90 (0.01)	
Risk group							
SR	336 (49)	0.84 (0.02)	0.81 (0.02)	**<0.001**	0.93 (0.02)	0.90 (0.02)	**0.003**
IR	259 (38)	0.85 (0.02)	0.84 (0.02)		0.93 (0.02)	0.92 (0.02)	
HR	93 (14)	0.67 (0.05)	0.67 (0.05)		0.80 (0.04)	0.80 (0.04)	
BM blasts day 15							
<5%	434 (77)	0.87 (0.02)	0.85 (0.02)	**<0.001**	0.93 (0.01)	0.93 (0.01)	**<0.001**
5-25%	101 (18)	0.73 (0.05)	0.72 (0.05)		0.91 (0.03)	0.87 (0.04)	
>25%	27 (4.8)	0.59 (0.10)	0.51 (0.11)		0.77 (0.09)	0.69 (0.11)	
BM blasts day 29							
<5%	609 (96)	0.84 (0.02)	0.82 (0.02)	**0.001**	0.93 (0.01)	0.91 (0.01)	**0.002**
5-25%	22 (3.5)	0.59 (0.11)	0.59 (0.11)		0.76 (0.09)	0.71 (0.10)	
>25%	3 (0.5)	0.67 (0.27)	nd		0.67 (0.27)	nd	

[1]Data missing for some variables: CNS disease (n = 7), mediastinal mass (n = 7), BM blasts at day 15 (n = 126), and BM blasts at day 29 (n = 54); [2]significant P values are in bold type. ALL: acute lymphoblastic leukemia; BM: bone marrow; CNS: central nervous system; HeH: high hyperdiploidy without t(1;19)(q21;q23), t(9;22)(q34;q11), der(11q23), or t(12;21)(p13;q22); HR: high risk; IR: intermediate risk; nd: not determined; pEFS: probability of event-free survival; pOS: probability of overall survival; SE: standard error; SR: standard risk; WBC: white blood cells.

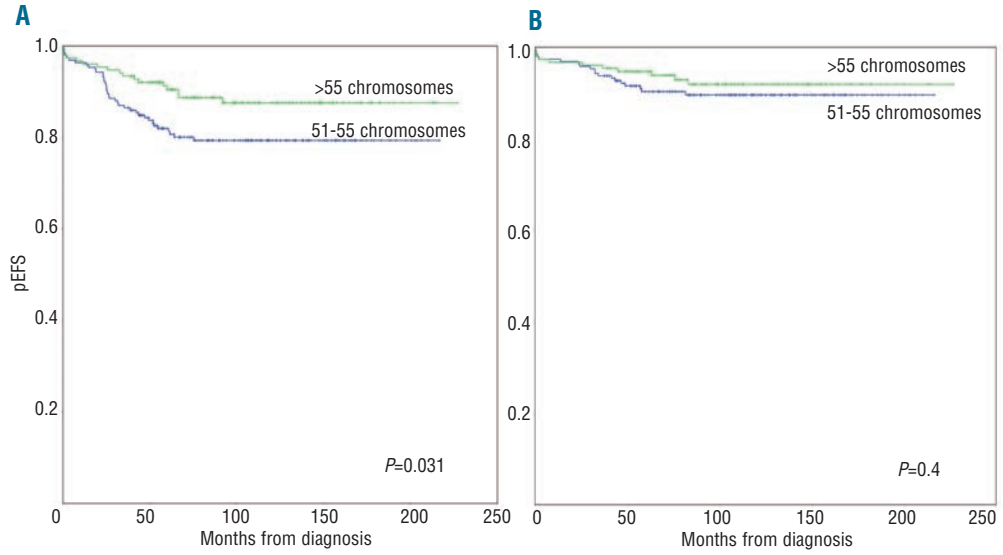

Figure 3. Event-free (A) and overall survival (B) of HeH cases by modal chromosome number. The pEFS and pOS analyses are based on the 344 patients informative for all the following parameters: age, WBC count, triple trisomies, and modal number.

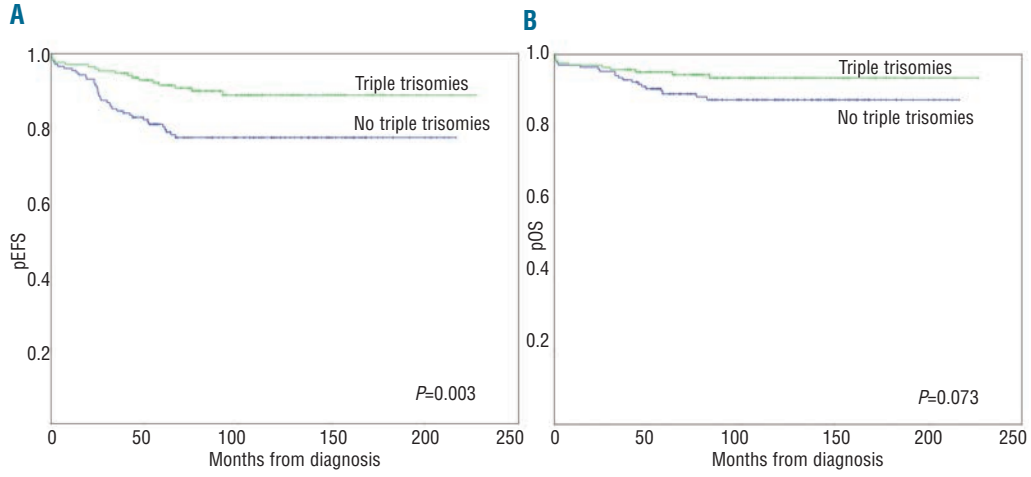

Figure 4. Event-free (A) and overall survival (B) of HeH cases by presence/absence of triple trisomies (+4, +10, +17). The pEFS and pOS analyses are based on the 344 patients informative for all the following parameters: age, WBC count, triple trisomies, and modal number.

fortuitous. The WBC counts were lower for cases with +4 than for cases disomic for this chromosome. Again, since no previous studies have reported such an association, this finding should be interpreted with caution. However, low WBC counts in HeH with gain of chromosome 4 would agree well with the significantly higher pEFS in such cases (*Online Supplementary Table S4*). As regards structural changes as such, they did not vary in relation to WBC counts or gender, nor did 1q gain, 6q deletion, or 17q gain. On the other hand, patients with HeH and structural changes were significantly older than those with only numerical changes. Interestingly, this is also the case when reviewing published HeH cases,[17] in which the median ages were 4 years for HeH patients with structural changes and 3 years for cases without. This would also be in line with a recent single nucleotide polymorphism array study of HeH showing that cases with 17q gain were older than those without such changes.[31] We also observed significant differences in age distributions for patients with +6, +17, and +18, with these trisomies being more common in younger children. In published HeH cases,[17] the median ages of trisomy-positive and disomy-positive cases for these three chromosomes are 3 and 4 years, respectively. Thus, trisomies 6, 17, and 18 are clearly typical for young age HeH. The biological implications of the observed age-related cytogenetic differences are, however, unknown.

Certain cytogenetic features were – in univariate analyses – also significantly associated with higher pEFS, namely + 4, +6, +17, +18, and +22, presence of triple trisomies, and high modal numbers, whereas structural changes as such, 1q gain, 6q deletion, and 17q gain were not associated with pEFS. However, no karyotypic patterns correlated with pOS (*Online Supplementary Table S4*). As regards the impact of certain gains on pEFS, our results agree, at least to some extent, with those of previous studies from the USA and the UK in which significantly higher pEFS were also observed among HeH patients with gains of chromosomes 4, 17, and 18; however, in contrast to our study, these gains also correlated with higher pOS in the UK series.[4,5,32] The triple trisomies +4, +10, and +17 were also associated with a favorable pEFS in this Nordic series, in line with COG data.[12] However, in agreement with UK trials,[7] the concurrent presence of these gains did not translate into superior OS (Figure 4). The finding of a better outcome, at least as regards pEFS, of HeH cases with higher modal chromosome numbers (Figure 3), with pEFS generally increasing with increasing modes (*Online Supplementary Table S2*), was not unexpected considering

several prior studies describing such an association.[2,4,5] Why high modal numbers would be a favorable prognostic factor is, however, enigmatic. One may speculate that lymphoblasts with high chromosome content are more susceptible to chemotherapy,[33] but this hypothesis awaits investigation.

The favorable impact of both triple trisomies and high modal numbers (*Online Supplementary Table S4*) suggests a close relationship between these two parameters. In fact, there is much support for this proposition. First, in our series, the modal chromosome numbers were significantly higher in triple trisomy-positive cases than in negative cases (*Online Supplementary Figure S1B and C*). Second, one could argue that the more chromosomes, the more triple trisomies, considering the quite restricted number of different frequent chromosome gains in HeH.[1,34] In fact, in the large series of HeH cases reviewed by Heerema *et al.*,[35] the frequencies of individual gains of chromosomes 4, 10, and 17 increased from 13-29% at mode 51 to 67-100% at mode 67. Thus, by necessity, triple trisomies must be more common at higher modes. Third, in the present multivariate analyses, low WBC count, young age, and high modal number were all associated with superior pEFS; the same was true for low WBC count, young age, and presence of triple trisomies. However, when including both modal number and triple trisomies, only WBC count remained statistically significant. The multicollinearity between high modal chromosome numbers and the presence of triple trisomies clearly proves that they are highly correlated. We, therefore, conclude that these two cytogenetic features identify the same subgroup of patients – an HeH cohort with a favorable prognosis. This begs the question: "which came first: the chicken or the egg?", triple trisomies or high modal numbers? Considering that there is ample evidence, based on a 2:2 allelic ratio of loci on tetrasomic chromosomes and few uniparental isodisomies in HeH cases, that the high hyperdiploid pattern usually arises as a simultaneous gain of chromosomes in one abnormal mitosis,[36-38] it seems most likely that the modal number is established first, with cases with high modal numbers also harboring several trisomies, including +4, +10, and +17. Thus, the triple trisomies are surrogate markers for high modal chromosome numbers and do not by themselves have a favorable prognostic impact.

In conclusion, in this population-based, consecutive Nordic series of 688 uniformly treated HeH cases, the most important factor for superior pEFS was low WBC count, with young age and high modal numbers/triple trisomies also being significantly associated with favorable outcome.

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