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ORIGINAL ARTICLE

The frequency and prognostic impact of dic(9;20)(p13.2;q11.2) in childhood B-cell precursor acute lymphoblastic leukemia: results from the NOPHO ALL-2000 trial

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The dic(9;20)(p13.2;q11.2) is reported to be present in ~2% of childhood B-cell precursor acute lymphoblastic leukemia (BCP ALL). However, it easily escapes detection by G-banding analysis and its true prevalence is hence unknown. We performed interphase fluorescence *in situ* hybridization analyses—in a three-step manner—using probes for: (i) *CDKN2A* at 9p21, (ii) 20p and 20q subtelomeres and (iii) cen9 and cen20. Out of 1033 BCP ALLs diagnosed from 2001 to 2006, 533 were analyzed; 16% (84/533) displayed 9p21 deletions, of which 30% (25/84) had dic(9;20). Thus, dic(9;20)-positivity was found in 4.7% (25/533), making it the third most common genetic subgroup after high hyperdiploidy and t(12;21)(p13;q22). The dic(9;20) was associated with a female predominance and an age peak at 3 years; 18/25 (72%) were allocated to non-standard risk treatment at diagnosis. Including cases detected by G-banding alone, 29 dic(9;20)-positive cases were treated according to the NOPHO ALL 2000 protocol. Relapses occurred in 24% (7/29) resulting in a 5-year event-free survival of 0.69, which was significantly worse than for t(12;21) (0.87; $P=0.002$) and high hyperdiploidy (0.82; $P=0.04$). We conclude that dic(9;20) is twice as common as previously surmised, with many cases going undetected by G-banding analysis, and that dic(9;20) should be considered a non-standard risk abnormality.
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Introduction

The chromosome abnormality dic(9;20)(p13. 2;q11.2) is a rare, but recurrent aberration in B-cell precursor acute lymphoblastic leukemia (BCP ALL); on the basis of G-banding analyses, the dic(9;20) has been reported to be present in 1–2% and 0.5% of childhood and adult BCP ALLs, respectively.[1–8] Since the dic(9;20) aberration was first described in 1995, (see refs. 1, 2) ~130 dic(9;20)-positive ALL cases have been reported in the literature.[9] However, despite this relatively high number, most publications on dic(9;20) have included small patient series, treated according to different protocols, precluding detailed analyses of the clinical impact of the aberration. To date, only six studies have comprised 10 or more dic(9;20)-positive cases.[2,4–8]

Studies on pediatric cases have shown that dic(9;20) positive leukemias display consistent BCP immunophenotypic features, with positivity for HLA-DR, CD10, CD19, CD20 and CD22 and negativity for T cell and myeloid markers, show a female predominance, and have a significant age incidence peak at 3–4 years. Most patients are allocated to non-standard risk treatment arms because of high white blood cell (WBC) count and a relatively high frequency of central nervous system (CNS) disease or other types of extra-medullary leukemia at diagnosis.[2,4–6] As regards, the prognostic implications and other clinical ramifications of dic(9;20), they remain to be clarified in detail.[5–7,10]

Several studies have characterized the dic(9;20) at the cytogenetic and molecular genetic levels, revealing breakpoint heterogeneity on both chromosomes, albeit with clustering of breaks in sub-bands 9p13.2 and 20q11.2. (see refs. 11–16) As this subtle abnormality easily escapes detection by conventional cytogenetics, being misclassified as monosomy 20 and/or deletion of 9p,[2–5] and that it may have a clinically important impact, other methods are definitely needed. As of yet, the dic(9;20) has not been shown to result in any gene fusion, rendering PCR-based methods unavailable for detection. Hence, fluorescence *in situ* hybridization (FISH) currently remains the only valid option to detect these cases. Metaphase FISH analyses with, for example, whole-chromosome painting probes for chromosomes 9 and 20, can be used successfully in many cases.[2–5] However, lack of methaphases or outgrowth of only normal cells during cell culture precludes metaphase FISH as a diagnostically reliable method. Array comparative genomic hybridization can also be used to detect the typical pattern of genomic imbalances associated with dic(9;20), (see refs. 13–16) but this technique can not be used to prove the dicentric nature of the rearrangement. Thus, at present, interphase FISH analyses are needed to identify all dic(9;20)-positive cases.

We have developed and validated a robust three-step interphase FISH method to identify the dic(9;20) rearrangement on diagnostic bone marrow (BM) smears, starting with

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identifying cases with loss of 9p, which is an inevitable consequence of the abnormality,[13–16] followed by investigating copy number imbalances between the p and q arms of chromosome 20 and co-localization patterns of the centromeres of chromosomes 9 and 20. By applying this FISH approach on a large Nordic pediatric series of BCP ALLs diagnosed between 2001 and 2006, and treated according to the NOPHO-2000 protocol,[17] we here show that the dic(9;20) is present in close to 5% of all cases, a frequency at least twice as high than the one based on G-banding analysis alone,[1–7] and that it is associated with a worse outcome (5-year event-free survival (EFS) and overall survival (OS) of 0.69 and 0.85, respectively) than standard risk patients.

Patients and methods

Patients

Between 1 January 2001 and 31 December 2006, 1174 infants, children and adolescents <18 years were diagnosed with ALL in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden). Among these, 1033 (88%) were BCP ALL, of which 882 (85%) were treated according to the NOPHO ALL 2000 protocol, which is described in detail elsewhere.[17] In short, risk stratification divided patients into standard intensity (SR: WBC $\geqslant 10 \times 10^9$/l, 1–9.9 years, no high risk (HR) features), intermediate intensity (IR: WBC $> 10 \times 10^9$/l, age >10 years, no HR features), and three HR groups (intensive, very intensive and extra intensive). HR features were WBC $\geqslant 100 \times 10^9$/l, 11q23/*MLL* rearrangement, t(9;22)(q34;q11), t(1;19)(q23;p13) and hypodiploidy (<45 chromosomes). The dic(9;20) was not a risk stratifying aberration in the NOPHO-ALL-2000 protocol.[17] Thus, the choice of treatment intensity for patients with dic(9;20)-positive ALL was made solely on the basis of age, WBC count, the presence of extra-medullary leukemia and morphologic response during induction therapy.

The NOPHO ALL 2000 protocol stipulated karyotyping and targeted analyses for 11q23/*MLL* rearrangement, t(1;19)(q23;p13) (*TCF3/PBX1*), t(9;22)(q34;q11) (*BCR/ABL1*) and t(12;21)(p13;q22) (*ETV6/RUNX1*). Diagnostic BM smears from 542 (52%) of the 1033 BCP ALL patients were sent to the Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden for interphase FISH investigations aimed at establishing the frequency and prognostic impact of 9p21/*CDKNA* deletions.[18] This study was subsequently developed further to include additional interphase FISH analyses on all 9p deleted cases to identify cases with dic(9;20); such analyses could be achieved in 533 of the cases. In the 491 BCP ALLs, which were not analyzed by interphase FISH for 9p21/*CDKNA* deletions, dic(9;20)-positive cases were ascertained using G-banding and metaphase FISH analyses only. A flowchart representing the various study cohorts analyzed in this study is shown in Figure 1.

The study was approved by the research ethics committee at Karolinska Institutet and informed consent was obtained in accordance with the Declaration of Helsinki.

Identification of dic(9;20)(p13.2;q11.2) by G-banding and metaphase FISH analyses

G-banding analyses were performed using standard methods in 15 cytogenetic laboratories in the Nordic countries, and all abnormal karyotypes were centrally reviewed. In suspected dic(9;20)-positive cases, where cells in fixative were available, additional metaphase FISH analyses, using CEP 9 and CEP 20 (Abbott Molecular Inc., Des Plaines, IL, USA) and/or WCP 9 and WCP 20 probes (Cytocell Ltd, Cambridge, UK), were performed to confirm the presence of dic(9;20).

Identification of dic(9;20)(p13.2;q11.2) by interphase FISH analysis

BM smears from 533 BCP ALL patients were analyzed in a three-step manner, first using probes to detect 9p deletions and then, if identified, probes for copy number imbalances between the p and q arms of chromosome 20 and co-localization patterns of the centromeres of chromosomes 9 and 20. The LSI *p16* (9p21) FISH probe (Abbott Molecular Inc.), representing a mixture of the *p16* (official gene symbol *CDKN2A*) probe labeled with Spectrum Orange and a CEP 9 probe labeled with Spectrum Green, was used for identifying 9p deletions. Cases with loss of *CDKN2A*, which all dic(9;20)-positive ALL cases have,[14–16] were subsequently screened, according to the manufacturer's instructions (Abbott Molecular Inc.), with the Vysis ToTelvysion probes that are specific for the subtelomeres of 20p and 20q. Cases displaying imbalances between the number of signals for 20p and 20q, that is loss of 20qter if one normal chromosome 20 was present together with the dic(9;20) and gain of 20pter if two normal chromosomes 20 were present together with dic(9;20), were further analyzed using CEP 9 and CEP 20 probes to confirm the presence of a dicentric rearrangement.

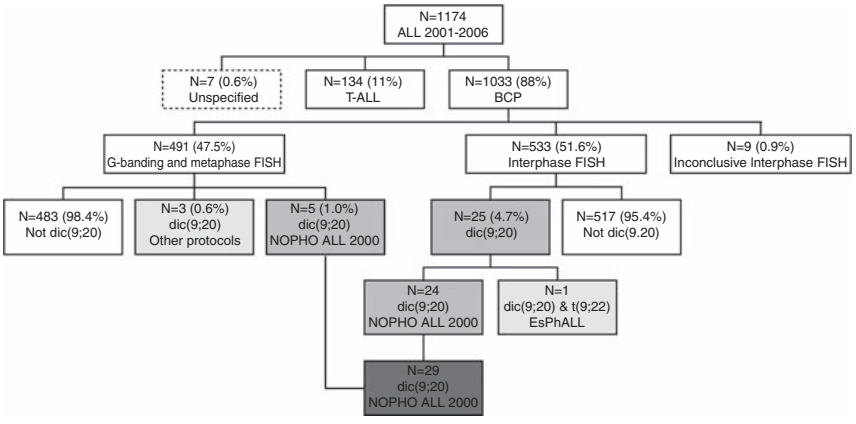

Figure 1 Flowchart of all 1174 childhood ALL cases (<18 years) diagnosed between 1 January 2001 and 31 December 2006, in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) depicting the study cohorts (indicated in yellow and green) analyzed in this report.

The reason why the centromeric probes were not used up-front was that initial analyses revealed a high false positive fusion rate in unselected cases and that normal controls showed a great variability in signal intensity of the CEP 20 probe.

The BM slides were pretreated, labeled, hybridized and washed according to the manufacturers' instructions (Abbott Molecular Inc.). The signals were analyzed using a Zeiss Axioskop 2 fluorescence microscope (Carl Zeiss, Göttingen, Germany) equipped with a cooled CCD camera (CoolSnap; Photometrics Ltd, Tucson, AZ, USA) controlled by a Power Macintosh computer (Apple Inc., Cupertino, CA, USA). Grey scale images were captured, pseudo-colored and merged using the SmartCapture 2 software (Digital Scientific Ltd, Cambridge, UK). In the vast majority, 200 nuclei were analyzed per sample.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software for Macintosh was used for the statistical analyses. The probabilities of EFS (pEFS) and OS (pOS) at 5 years after diagnosis were calculated using the Kaplan–Meier method and the different cytogenetic subgroups listed in Tables 1 and 2 were compared using the log rank test. A multivariate analysis—including all cytogenetically defined subgroups as well as clinical parameters (WBC and age at diagnosis)—was also performed. The significance limit for two-sided *P*-values was set to <0.05 in all tests. Time in first remission was defined as time from diagnosis until first event, comprising induction failure, relapse, death of disease, death in remission, or second malignant neoplasm. In the OS analysis, death of any cause was the endpoint. The median observation time for patients in continuous complete first remission was 67 months (range 28–108 months). The NOPHO leukemia registry is updated annually and follow-up data were extracted from the registry on 1 April 2010.

Results

Interphase FISH results

The clinical characteristics (WBC, gender and extra-medullary leukemia) and the frequency distribution of the various genetic subgroups except the dic(9;20) group (Table 1) of the 533 BCP ALL cases with available slides for interphase FISH analyses for dic(9;20) did not differ significantly from the 491 BCP ALL cases diagnosed during the same time period from which no BM smears were provided for analysis (data not shown).

Deletions of *CDKN2A* (9p21) were detected in 84 (16%) of the 533 cases, with 27 (5%) being homozygous and 57 (11%) hemizygous. Further FISH studies for dic(9;20) revealed that 25 (30%) of the 84 cases with deletion of 9p harbored dic(9;20), of which 19 (76%) displayed a FISH probe pattern consistent with one normal chromosome 20 present together with the

Table 1 Clinical characteristics, risk classification and genetic subdivision of the 533 BCP ALLs analyzed for dic(9;20) by interphase FISH analysis

Features	*HeH*	*t(12;21)**	***dic(9;20)*#***	*t(1;19)*	*11q23/MLL*	*t(9;22)#*	*iAMP21*	*<45 chr*	*Normal*	*Others*	*Failure*
No. of cases (%)	181 (34)	120 (23)	**25 (4.7)**	18 (3.4)	19 (3.6)	12 (2.3)	10 (1.9)	7 (1.3)	56 (11)	66 (12)	19 (3.6)
Gender											
Female (%)	82 (45)	56 (47)	**17 (68)**	11 (61)	9 (47)	4 (33)	2 (20)	5 (71)	25 (45)	28 (42)	7 (37)
Male (%)	99 (55)	64 (53)	**8 (32)**	7 (39)	10 (53)	8 (67)	8 (80)	2 (29)	31 (55)	38 (58)	12 (63)
Age (years)											
Max	17.9	15.6	**15.1**	13.1	13.1	17.9	17.3	12.8	14.8	17.7	14.4
Median	3.7	4.5	**3.3**	4.3	1.0	9.1	9.7	4.1	4.9	6.4	6.7
Min	1.0	1.4	**1.3**	1.9	0.1	4.0	3.1	3.0	1.0	1.4	1.6
WBC count (x 10^9/l)											
Max	171	260	**336**	159	528	101	30.7	100	170	172	231
Median	7.0	7.9	**19.2**	34.7	76.0	15.1	3.2	15.7	12	13.4	12.3
Min	0.6	0.9	**1.6**	4.1	3.1	1.3	1.3	3.9	0.3	0.8	1.7
EML (%)	6 (3.3)	3 (2.5)	**1 (4.0)**	0	2 (11)	1 (8.3)	0	0	1 (1.8)	0	0
Classification											
SR (%)	94 (52)	56 (47)	**7 (28)**	1 (5.6)	1 (5.3)	0	3 (30)	0	13 (23)	15 (23)	6 (32)
IR (%)	61 (34)	41 (34)	**5 (20)**	1 (5.6)	1 (5.3)	0	6 (60)	0	27 (48)	34 (52)	5 (26)
HR (%)	24 (13)	23 (19)	**12 (48)**	16 (89)	7 (37)	4 (33)	1 (10)	7 (100)	14 (25)	16 (24)	8 (42)
Other (%)	2 (1.1)	0	**1 (4.0)**	0	10 (53)	8 (67)	0	0	2 (3.6)	1 (1.5)	0
Primary events											
CR1 (%)	150 (83)	105 (88)	**17 (68)**	15 (83)	10 (53)	8 (67)	5 (50)	4 (57)	49 (88)	57 (86)	14 (74)
Induction failure (%)	2 (1.1)	0	**0**	0	1 (5.3)	0	0	0	0	1 (1.5)	1 (5.3)
Resistant disease (%)	2 (1.1)	0	**0**	0	0	0	0	0	1 (1.8)	1 (1.5)	0
Relapse (%)	19 (10)	13 (11)	**6 (24)**	3 (17)	8 (42)	4 (33)	5 (50)	1 (14)	4 (7.1)	7 (11)	4 (21)
Dead in CR1 (%)	5 (2.8)	0	**1 (4.0)**	0	0	0	0	2 (29)	0	0	0
SMN	3 (1.7)	2 (1.7)	**1 (4.0)**	0	0	0	0	0	2 (3.6)	0	0

Abbreviations: BCP ALL, B-cell precursor acute lymphoblastic leukemia; chr, chromosomes; CR1, complete first remission; EML, extra-medullary leukemia at diagnosis (spread to central nervous system and/or mediastinum and/or testis); FISH, fluorescence *in situ* hybridization; HeH; high hyperdiploidy (defined as 51–67 chromosomes); HR, high risk; iAMP21, intrachromosomal amplification of chromosome 21; IR, intermediate risk; SMN, second malignant neoplasm; SR, standard risk; WBC, white blood cell.
In this table, *one case with dic(9;20) and t(12;21) and #one with dic(9;20) and t(9;22) are included in the dic(9;20) group. The classification 'Other' includes patients treated according to the Interfant-99, (see ref. 18) EsPhALL, or modified BFM.[21] dic(9;20)-positive cases are marked in bold.

Table 2 Survival after a median follow-up of 67 months among the 882 children with BCP ALL treated according to the NOPHO ALL 2000 protocol, divided into the main cytogenetic subgroups

Subgroup	*No. of cases (%)*	*pEFS (SE)*	*pOS (SE)*	P-*value*[a] *(EFS)*	P-*value*[a] *(OS)*
HeH	295 (33)	0.82 (0.02)	0.92 (0.02)	0.04	0.19
t(12;21)[b]	188 (21)	0.87 (0.03)	0.97 (0.01)	0.002	0.002
dic(9;20)[b]	29 (3.3)	0.69 (0.09)	0.85 (0.07)	—	—
t(1;19)	30 (3.4)	0.73 (0.08)	0.77 (0.08)	0.78	0.31
11q23/*MLL*	18 (2.0)	0.56 (0.12)	0.61 (0.11)	0.14	0.03
t(9;22)[b]	11 (1.2)	0.36 (0.15)	0.72 (0.14)	0.05	0.35
iAMP21	12 (1.4)	0.50 (0.14)	0.81 (0.12)	0.54	0.88
<45 chr	10 (1.1)	0.50 (0.16)	0.56 (0.17)	0.18	0.08
Normal	112 (13)	0.85 (0.03)	0.93 (0.03)	0.05	0.36
Others	123 (14)	0.80 (0.04)	0.88 (0.03)	0.13	0.74
Failure	54 (6.1)	0.76 (0.06)	0.84 (0.05)	0.46	0.75
Total	882 (100)				

Abbreviations: BCP ALL, B-cell precursor acute lymphoblastic leukemia; chr, chromosomes; HeH, high hyperdiploidy (defined as 51–67 chromosomes); iAMP21, intrachromosomal amplification of chromosome 21; pEFS, probability of event-free survival; pOS, probability of overall survival; SE; standard error.
[a]The *P*-values given indicate pairwise comparisons with dic(9;20).
[b]The case with dic(9;20) and t(12;21) is included in the dic(9;20) group, whereas the one with dic(9;20) and t(9;22) is included in the t(9;22) group.

dic(9;20) and 6 (24%) displayed a pattern indicating the presence of two normal chromosomes 20 together with the dic(9;20). Taken together, the dic(9;20) was found in 4.7% (25/533) of all BCP ALL cases included in the study.

The dic(9;20) was the 'primary' change in all cases except two: one also harbored a t(9;22)(q34;q11) and one also had a t(12;21)(p13;q22) (Tables 1 and 2); both these translocations were confirmed by reverse transcription-PCR and/or FISH.

Chromosome banding and metaphase FISH results

In the whole cohort of 882 patients treated according to the NOPHO ALL 2000 protocol, targeted FISH and/or PCR analyses revealed *MLL* rearrangements in 16 of 596 investigated cases, *TCF3/PBX1* in 28/504 cases, *BCR/ABL1* in 9/670 cases and *ETV6/RUNX1* in 188/745 cases. G-banding analysis, which was performed in all cases, showed two additional cases each with 11q23 rearrangements, t(1;19) and t(9;22) (Table 2).

Apart from the 25 cases with dic(9;20) detected by interphase FISH, eight additional dic(9;20)-positive BCP ALL cases, diagnosed during the same time period, were identified by G-banding and metaphase FISH analyses among the 491 BCP ALLs that were not included in the interphase FISH study (Figure 1).

Among the 25 dic(9;20)-positive cases identified by interphase FISH, only 11 (44%) had been detected by G-banding/metaphase FISH analyses. In these 11 cases, the dic(9;20) was the sole change in 3, whereas 8 cases had additional chromosome abnormalities, of which +20 and +21 were recurrent. Nine of these 11 cases were reported previously.[5] In the remaining 14 cases, 2 were cytogenetic failures, 3 had a normal karyotype and 9 had additional changes, with −9, −20 and +21 being recurrent.

In total, among the 20 cytogenetically informative cases ascertained through interphase FISH analyses, additional chromosomal abnormalities were found in 15/20 (75%), with 5/20 (25%) harboring numerical changes only and 10/20 (50%) displaying both structural and numerical aberrations. The majority (15/20; 75%) had only unbalanced changes, whereas 5/20 (25%) had both unbalanced and balanced aberrations. Four recurring numerical changes were detected; −20 in 14/20 (70%) (indicating that only one normal chromosome 20 is present as the centromere of that chromosome present in the dic(9;20)), +21 in 8/20 (40%), +20 in 6/20 (30%) and −9 in 2/20 (10%). Among the 10 cases with additional structural changes, 5 harbored balanced translocations: t(2;12)(q11;p11), t(5;12)(p13;q15), t(9;22)(q34;q11), t(11;17)(q21;q23) and t(12;21)(p13;q22).

Survival

Of the 25 cases diagnosed as dic(9;20)-positive by interphase FISH analysis, one patient was excluded from the survival analysis because of the presence of t(9;22); this patient did not follow the NOPHO ALL 2000 protocol, but was treated according to the ongoing EsPhALL protocol. The dic(9;20)-positive case that also harbored a t(12;21) was, however, included in the survival analysis as the latter aberration was not risk-stratifying in the NOPHO ALL 2000 protocol (Figure 1).

Of the eight patients with dic(9;20)-positive ALL identified by G-banding/metaphase FISH alone, one was excluded from the survival analysis because of age <1 year; this patient was treated according to the Interfant99 protocol.[19] Two patients were excluded as their treatment followed the NOPHO ALL 1992 protocol (Figure 1).

Thus, 29 (24 + 5; Figure 1; Table 2) cases treated according to the NOPHO ALL 2000 protocol were available for analysis as regards EFS and OS. To date, 7/29 patients (24%) have relapsed, four in the BM (after 7 (HR—initial risk group), 13 (HR), 30 (HR) and 33 (HR) months, respectively) and three with isolated CNS relapses (after 22 (IR), 25 (IR), and 29 (SR) months, respectively). In total, 3/7 (43%) dic(9;20) positive cases had isolated CNS relapses while the corresponding frequency for the whole cohort of 882 patients was 22/135 (16%), when including cases with dic(9;20), or 19/128 (15%) when the dic(9;20)-positive cases were excluded, a statistically non-significant difference. Furthermore, one patient died in complete first remission after 12 (HR) months because of infection and one developed a second malignancy (non-Hodgkin lymphoma) after 30 (SR) months. Hence, in total, nine primary events have occurred, giving a pEFS at 5 years of 0.69 (Table 2). The pEFS at 5 years for the main cytogenetic subgroups of all the 882 BCP ALL cases diagnosed during the study period and treated according to NOPHO ALL 2000 are shown in Figure 2a and listed in Table 2. Paired univariate analyses showed a significant worse outcome in first remission for dic(9;20)-positive cases compared with

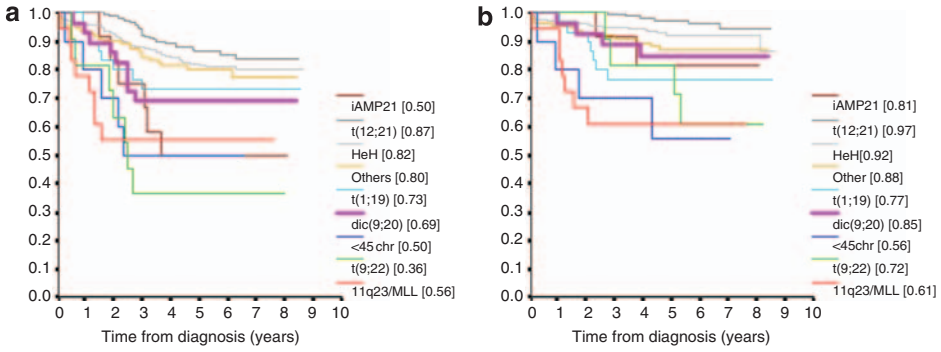

Figure 2 (**a** and **b**) Kaplan–Meier survival plots comprising the 882 BCP ALL cases included in the study and treated according to the NOPHO ALL 2000 protocol, demonstrating the (**a**) pEFS and (**b**) pOS for cases with dic(9;20) and for cases belonging to the other main cytogenetic subgroups (Table 2). Cases with normal karyotypes and failures are not shown.

t(12;21)- and HeH-positive cases (pEFS = 0.87; $P = 0.002$ and pEFS = 0.82; $P = 0.04$, respectively). In a Cox multivariate analysis—including the different cytogenetic subgroups, WBC and age, and using the dic(9;20) cases as reference group—only the t(12;21) positive cases came out as having a statistically superior outcome ($P = 0.01$). WBC is still the most powerful predictor of EFS ($P < 0.01$).

Four of the 29 children with dic(9;20)-positive BCP ALL have died (one in complete first remission and three from leukemia) giving a pOS at 5 years of 0.85 (Table 2). The pOS at 5 years for the different cytogenetic subgroups in the cohort of 882 BCP ALLs treated following the NOPHO ALL 2000 protocol are shown in Figure 2b and listed in Table 2. A paired univariate analysis showed a significantly worse pOS for dic(9;20)-positive cases compared with those having t(12;21) (pOS = 0.97; $P = 0.002$). A multivariate analysis—including the cytogenetic subgroups, WBC and age at diagnosis—revealed WBC and age to be the best predictors of OS ($P < 0.01$); the only cytogenetic abnormality still having a significant prognostic impact was t(12;21) ($P = 0.035$).

Discussion

The salient results of the present study are that: (1) dic(9;20) occurs in ~5% of childhood BCP ALL, a frequency at least twice as high as previously reported based on chromosome banding analyses alone; (2) approximately 25% of all dic(9;20)-positive cases escape G-banding detection if only monosomy 20 is used as a pointer to dic(9;20), as 1/4 of the cases harbors two normal chromosomes 20 in addition to the dic(9;20); (3) it is possible to detect the aberration on BM smears using a three-step FISH approach; (4) the dic(9;20) subgroup comprises 1/3 of all BCP ALL with deletion of 9p21; and that (5) dic(9;20) should be considered a non-standard risk aberration.

Previous studies on the incidence of dic(9;20) in ALL were all based on conventional chromosome banding analyses,[1–7,10] often supplemented with metaphase FISH analyses, in particular in cases with 'monosomy 20', which is a well-known pointer to the presence of dic(9;20).[4] On the basis of such studies, the frequency in childhood ALL has been reported to be 0.7–2.5%. (see refs. 4–8) However, this is undoubtedly an underestimate, partly because of the bias of preferentially focusing on cases displaying monosomy 20. We have previously shown that some dic(9;20)-positive cases harbor two normal chromosomes 20 in addition to the dic(9;20). (see ref. 15) Hence, identifying loss of chromosome 20 is insufficient to ascertain all cases with dic(9;20). Furthermore—and as clearly seen in the present study—a substantial proportion of cases go undetected because of uninformative cytogenetic results. Thus, we decided to develop a robust FISH method to detect cases also on the interphase level, using a three-step approach. By applying this method, we identified not only all cases found by G-banding and metaphase FISH analyses, but also several additional cases. In total, close to 5% of all childhood BCP ALLs were shown to harbor the dic(9;20), making it the third most common cytogenetic subgroup after high hyperdiploidy and t(12;21) (Table 1).

As several groups have reported that the dic(9;20) can occur together with other prognostically important cytogenetic aberrations it may be questioned whether dic(9;20) should be considered a 'primary' chromosome rearrangement or not.[5–7] Apart from the t(9;22)-positive case in this report, the t(9;22) has been reported in association with dic(9;20) in five previous cases—three adult[1,10,20] and two pediatric BCP ALLs.[6,13] Furthermore, in the only dic(9;20)-positive T-cell ALL reported to date, a t(8;14)(q24;q11) was also present.[4] To the best of our knowledge, the coexistence of t(12;21) and dic(9;20), as in one of the present cases, has never been reported previously. Taken together, it seems safe to conclude that although dic(9;20) may be a 'primary' change one should definitely not exclude the presence of additional 'primary' changes, such as t(12;21) and t(9;22), with at least the latter abnormality having major clinical ramifications. Further studies are needed to elucidate whether the molecular genetic outcome of the dic(9;20) differs between cases with or without other 'primary' changes. This notwithstanding, it is of great importance to keep the possibility of other 'primary' aberrations in mind if a hierarchical strategy is used for FISH and/or molecular analyses in the genetic diagnosis of BCP ALL.

The previously reported female predominance[5,6] was also observed in the present study where the female/male ratio was 2.1 (Table 1). Such a pronounced gender difference is otherwise quite unusual in BCP ALL, in particular as regards a preponderance of girls (Table 1). Other previously recognized clinical features of BCP ALL with dic(9;20) seen in this series include young age (median 3.3 years), somewhat high WBC counts (median 19.2×10^9/l), and frequent (72%) non-standard risk allocation on the basis of traditional risk criteria (Table 1). Thus, the patient cohort identified herein does not seem to differ from previously reported series and there is hence no obvious inclusion bias that should be taken into account when analyzing the clinical outcome of the patients in relation to previously published survival studies.

Most children with BCP ALL can be cured,[7,21] but the outcome is poor for the minority of patients who do relapse during or after treatment. Our study and several other reports[3,4,6,7] strongly indicate that a substantial proportion (16–25%) of the dic(9;20)-positive cases relapse and that there is marked risk for CNS relapse. In fact, in the present series, comprising of 29 dic(9;20)-positive cases strictly treated according to the NOPHO-ALL 2000 protocol,[17] pEFS at 5 years (0.69) was significantly lower than for the traditional standard risk genetic subgroups high hyperdiploidy and t(12;21) (Table 2). Although a second remission often could be achieved, leading to a pOS of 0.85, there was still a significantly worse outcome compared with t(12;21)-positive cases (Table 2). All CNS relapses occurred in children treated according to the SR or IR arms using traditional maintenance with methotrexate and 6-mercaptopurin orally, whereas all BM relapses occurred in HR patients receiving maintenance according to the LSA_2L_2 protocol (an intensive block-based therapy originally intended for treating B-cell non-Hodgkin lymphoma).[21] This may suggest that traditional maintenance is a better choice for dic(9;20)-positive cases. Previous studies have yielded somewhat conflicting data as regards the prognostic impact of dic(9;20). Hereema *et al.*[8] reported an overall poorer outcome for patients with a 9p abnormality compared with those without, and did not find any significant prognostic differences between cases with dic(9;20) and cases with other 9p abnormalities. In a recent study,[6] 19 dic(9;20)-positive BCP ALL cases treated according to the Berlin-Frankfurt-Münster (ALL-BFM) protocols[22] had a rather favorable outcome, with a lower relapse rate (16%) and a higher 5-year EFS rate (75%) and OS (94%) compared with the outcome (29, 62 and 82%, respectively) for the NOPHO patients previously reported by Forestier *et al.*[5] and for the present NOPHO patient cohort (24, 69 and 85%, respectively). However, four patients had CNS involvement at diagnosis and there was a tendency for CNS disease in case of relapse.[6] Moorman *et al.*[7] reported that the 13 dic(9;20)-positive cases included in the UK Medical Research Council ALL97/99 did not show evidence of an increased risk of relapse or worse outcome with pEFS and pOS at 5 years of 77 and 92%, respectively; however, they nevertheless included the aberration in the intermediate risk group. These differences may perhaps be explained by different efficacy of the different treatment protocols used. Indeed, we have previously shown, using *in vitro* studies, a high cellular sensitivity of dic(9;20)-positive blasts to L-asparaginase (L-Asp) and cytarabine (Ara-C).[23] In the ALL-BFM and MRC protocols, higher doses of L-Asp are given for longer time periods starting during induction, whereas the NOPHO protocol makes use of smaller doses at fewer time points and strictly post-induction. The strategies for Ara-C treatments are largely similar in the three protocols.[17,22,24] Thus, one could speculate that these drugs, when given as in the BFM protocol, may contribute to the more favorable outcome seen in the BFM study.[6] If correct, it becomes even more important to identify all dic(9;20)-positive cases using an interphase FISH strategy in order to assign the patients to correct risk groups and proper treatment regimens.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

VZ performed research, analyzed data and wrote the paper. FG performed research and analyzed data. EK and JS performed research. MH identified specimens, provided clinical data, and analyzed data. GL, EB, GB, IG, BG, HE, LC, MN and LP analyzed data. EF and BJ analyzed data and wrote the paper. AN, principal investigator, designed research, analyzed data and wrote the paper.

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