An investigation into whether deletions in 9p reflect prognosis in adult precursor B-cell acute lymphoblastic leukemia: a multi-center study of 381 patients

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ABSTRACT

In acute lymphoblastic leukemia, besides age and white cell count at diagnosis, the cytogenetic abnormalities t(9;22)/BCR-ABL and t(4;11)/MLL-AF4 are important prognostic markers and are often included in the treatment stratification of patients with adult acute lymphoblastic leukemia. Deletions in 9p are seen in about 9% of cases of adult acute lymphoblastic leukemia, but their prognostic impact has been controversial. Cytogenetic data from 381 patients diagnosed with B-precursor acute lymphoblastic leukemia were reviewed. Chromosomal analysis was successful in 240 cases. Of these cases, 18 (8%) had abnormalities in 9p and they were compared with patients with normal karyotypes and patients with t(9;22)/BCR-ABL. Patients with abnormalities of chromosome 9 showed significantly shorter overall survival compared with patients with normal karyotypes. In fact, overall survival was similar to that in the poor prognosis t(9;22)/BCR-ABL-positive group. Our data suggest that chromosomal abnormalities involving 9p may have a significant negative impact on survival in adult B-precursor acute lymphoblastic leukemia.

Key words: p14ARF, p53, 9p21, leukemia.

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Introduction

In acute lymphoblastic leukemia (ALL), several chromosomal aberrations with an impact on prognosis have been described, such as t(9;22), t(4;11), t(1;9), and hyperdiploid or hypodiploid karyotype. Besides t(9;22)(q34;q11)/BCR-ABL and t(4;11)(q21;q23)/MLL-AF4, an elevated white blood cell count, age over 40 and non-responders/slow responders to chemotherapy are commonly regarded as high risk criteria in ALL.[1] Twenty to thirty per cent of all adults with ALL have been found to have a normal karyotype.[2] The oncogene BCR-ABL occurs in approximately 3% of all cases of childhood ALL and in 25–30% of cases of adult ALL. Deletions in 9p are found in about 9% of cases of adult ALL.[4] The 9p21 region encodes the tumor suppressor genes *p16INK4a*, *p14ARF* and p15INK4b. Most studies of 9p anomalies and molecular analyses of *p16INK4a*, *p14ARF* and *p15INK4b* have been performed in connection with childhood ALL. The prognostic impact of deletions in 9p has been controversial.[2-4]

Treatment of adult ALL is often adapted according to risk group (standard or high risk). Allogeneic bone marrow transplantation (BMT) has been included in the post-remission treatment of ALL subgroups with a high risk of relapse.[5,6] Allogeneic BMT is considered to be superior to maintenance chemotherapy in BCR-ABL-positive ALL[7] and is often preferred in other high risk groups, but it is not usually a standard treatment in patients with del(9p). In this retrospective analysis of 381 adult B-precursor ALL patients, we show that abnormalities in chromosome 9 (-9p/-9) were present in 8% of the patients. We demonstrate that patients with abnormal 9p show significantly inferior overall survival (OS) when compared with those with normal karyotypes. Moreover, we show that there was no difference in OS between patients with t(9;22) and those with abnormal 9p.

Design and Methods

Patients

Adult patients diagnosed with B-precursor ALL in Sweden and reported to the Swedish Adult ALL Group in 1986-2000,

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Table 1. Characteristics of 240 patients defined by cytogenetic analysis, *patients investigated with FISH/PCR for the presence of a BCR/ABL rearrangement, all positive patients are under the t(9;22) category.

		cases (%) N.	*OS 3-year (95% CI)*	*Age y Mean (range)*	*WBC count (x10⁹/L) Mean (range)*	*CNS leukemia*	*dead*	*alive*	*Bcl/abl**
9p/-9		(7.5) 15	20(0-40)%	48 (21-77)	34 (0-280)	0	13	2	10
	BMT	6	33 (0-71)%	33.8 (21-57)	8 (0-20)	0	5	1	
	No BMT	9	11 (0-32)%	55.2 (26-77)	54 (1-280)	0	8	1	
-9p/-9 and t(9;22)		3	0	54(43-61)	80(38-130)	1	3	0	3
Normal		(23.3) 56	52 (38-66)%	37 (17-74)	42 (1-308)	1	28	28	38
	BMT	17	48 (24-72)%	28 (21-46)	47 (1-308)	1	10	7	
	No BMT	39	56 (38-73)%	42 (17-74)	40 (1-249)	0	18	21	
t(9;22)		(33) 78	23 (14-33)%	44 (19-78)	53 (2-860)	9	64	14	69
	BMT	48	36(22-49)% (19-59)	38 (2-258)	37	6	35	13	
	No BMT	30	3(0-10)%	56 (29-78)	78 (2-860)	3	29	1	
t(4;11)		(3.8) 9	33 (3-64)% (17-72)	33 (1-338)	137	0	6	3	5
	BMT	5	40 (10-83)%	25.3 (17-33)	177 (46-338)	0	3	2	
	No BMT	4	25 (0-67%)	42 (22-72)	71 (1-130)	0	3	1	
HH**		(11.6) 28	54 (31-69)%	41 (18-74)	19 (1-111)	3	14	14	22
	BMT	11	53(23-83)%	33 (20-57)	27 (2-111)	3	7	4	
	No BMT	17	55(31-80)%	47 (18-74)	14 (1-55)	0	7	10	
Complex		(3.8) 7	0	48 (20-79)	193 (6-380)	0	7	0	5
-17		(2) 5	20 (0-55)		30(3-83)	0	5	0	2
t(1;19)		(1) 3	67 (13-100)%		96(13-257)		2	1	1
t(8;14)		(1) 3	33 (0-87%)		11(6-16)	0	1	2	1
Other		(13) 33	23 (9-38)%		52(1-600)	0	6	27	24
Total		(100) 240	33 (27-40)%	42 (17-81)	50 (0-860)	14	142	98	180

***HH: high hyperdiploidy (>50 chromosomes).*

and in addition those diagnosed at Karolinska University Hospital Huddinge in 2001-2006, were investigated. The study included a total of 381 patients aged 17-78 years. Diagnosis of B-precursor ALL was made according to French-American-British (FAB) and World Health Organization (WHO) classification when this was available. The majority of the patients were treated uniformly according to Swedish national protocols. Between 1986 and 1993, the national treatment protocol consisted of traditional ALL treatment adopted from the L-10 protocol.[8,9] From 1994, the national treatment protocol consisted of an intensive chemotherapy regimen with high-dose cytarabine upfront.[10] One hundred and fifty-five (41%) patients underwent allogeneic stem cell transplantation either upfront, if the patient was considered to have a high risk leukemia (defined as t(9;22)/BCR-ABL, t(4;11), white blood cell count above 30×10⁹/L, CNS leukemia

or late remission), or as salvage treatment in second complete remission (CR2) and beyond CR2.

Chromosomal analysis

Cytogenetic analyses were successful in 240 cases. In 87 patients the analysis was considered inadequate (no mitoses or fewer than 5 metaphases in the absence of an abnormal clone) and in 54 patients (14%) no analysis was performed. Thus, 240 (63%) out of 381 patients were eligible for this study. In 273 cases, reverse-transcription polymerase chain reactions (RT-PCRs) or fluorescence *in situ* hybridization (FISH) analyses were carried out in order to detect the *BCR-ABL* fusion gene. Patients with five or more chromosome aberrations were considered to have a complex karyotype. High hyperdiploidy was defined as > 50 chromosomes.

Statistical analysis

Overall survival was defined as the time from diagnosis to death from any cause. It was estimated by the Kaplan-Meier method and differences between subtypes were tested using the log-rank test.[11] Prognostic factors for OS were evaluated by univariate and multivariate analyses and the Cox proportional hazard regression method. p values <0.05 were considered significant for all tests.

Results and Discussion

Patients' characteristics are listed in Table 1. The mean age was 42 (range 17–81) years and 83 patients (35%) were below the age of 40. The most common aberration was t(9;22), which was found in 78 patients (33%). The mean age of this group was 44 years (19–78), of whom 35 (45%) were below the age of 40. Sixty-two per cent of all the patients with t(9;22) had undergone BMT, 4 of them in CR2 and all the others upfront. The mean time to transplantation was five months. Fifty-six patients had normal karyotypes and 17 of these (31%) had undergone BMT, 8 in first remission because of a high white blood cell count (n=7) or CNS (n=1) involvement. The mean time to transplantation was 15 months. High hyperdiploidy was found in 28 patients and 11 (40%) of these had undergone BMT.

In 18 patients, including 7 under 40 years of age, we found either del(9)(p21), -9 or translocations involving 9p (Table 1). Monosomy 9 was found in 3 cases, with the following karyotypes: 45,XX,-9, 43,XX,-2,-9,-14,t(9;22)(q34;q11) and 47,XY,+6,-9,+19. Translocations involving 9p were found in 2 cases, 46,XY, t(3;9)(p21;p22),t(9;22)(q34;q11) and 46XY,t(9;9)(p21;p23-24). In 3, including the 2 patients described above together with one additional patient, of these 18 patients the chromosomal analysis also revealed t(9;22). Six of these 18 patients (33%) had undergone transplantation, one upfront and 5 in CR2 or beyond. The mean time to transplantation was 23 months.

Of the common cytogenetic groups, inferior outcome was seen in the patients that had chromosome 9 aberrations. The 3-year overall survival rate in this group was only 13% (95% CI 0–30%), 20% (95% CI 0–40%) excluding patients with both t(9;22)/BCR-ABL and abnormal 9p. Only 2 of the 18 patients were still alive, one of whom had undergone BMT. Patients with t(9;22)/BCR-ABL had a similar outcome, with a 3-year OS rate of 22% (95% CI 12–31%). However, all but one of the 16 patients still alive had undergone BMT. None of the 3 patients with both t(9;22)/BCR-ABL and abnormal 9p are alive, they died after four, nine and 14 months. These 3 patients were excluded from further analyses regarding the 9p and t(9;22)/BCR-ABL groups.

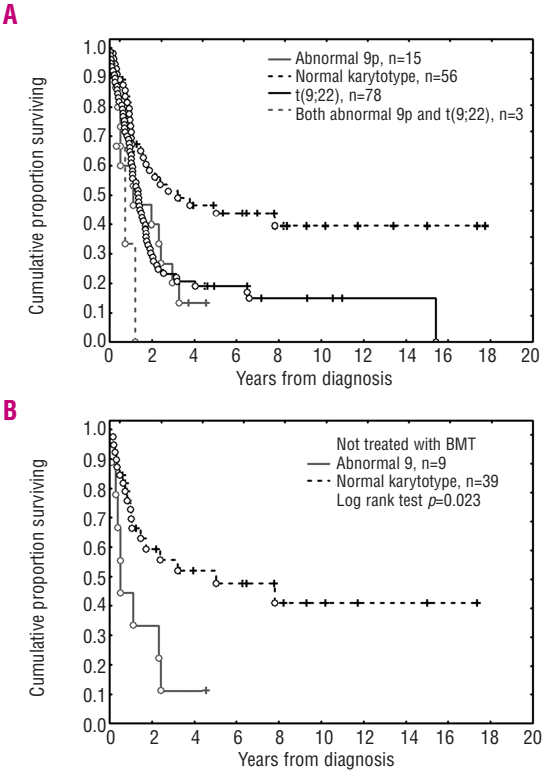

Figure 1. Overall survival – univariate analysis. (A) All patients with normal karyotype compared with patients with abnormal chromosome 9, p=0.007, and all patients with t(9;22) compared with patients with abnormal chromosome 9, p=0.781. (B) Patients with normal karyotype *vs.* those with abnormal chromosome 9 who did not undergo BMT.

Comparison of the group with abnormal 9p with those with normal karyotype showed a significantly shorter survival period among those with abnormal 9p (p<0.01, log-rank test, Figure 1A). Excluding patients with translocation involving 9p21 (n=2) and cases with both t(9;22)/BCR-ABL and abnormal 9p (n=3), the difference was still significant (p=0.020). The median event free survival in patients with normal karyotype was 2.5 years, while in patients with abnormal 9p it was only six months, (p=0.0134). Patients with normal karyotype and who were not treated with BMT had a median OS of five years, but it was only five months in patients with abnormal 9, (p=0.023, Figure 1B). Patients with t(9;22) had a significantly worse outcome compared with those with normal karyotype (p<0.01). A Cox proportional hazard model was used

Table 2. Cox regression model concerning the risk of death among patients with chromosome 9 deletion, t(9;22)/BCR-ABL and normal karyotype.

	Variable	*RR (95% CI)*	*p*
Karyotype (normal)	Del 9p	2.21 (1.07–4.57)	0.032
	t(9;22)/BCR-ABL	1.95 (1.16–3.29)	0.012
Age (<40 years)	≥40 years	2.11 (1.38–3.23)	<0.001
Sex (male)	female	0.77 (0.50–1.16)	0.21
White blood cell count ($<30\times10^9$/L)	$\geq30\times10^9$/L	1.51 (0.98–2.34)	0.065

to analyze the prognostic impact of chromosome 9 deletions and t(9;22)/BCR-ABL versus normal karyotype. In the first model, only the karyotype was analyzed and both chromosome 9 deletions and t(9;22)/BCR-ABL were found to be significant negative predictors as regards OS. In the multivariate analysis, age, sex and white blood cell count were analyzed together with karyotype. Age over 40 years, t(9;22)/BCR-ABL and chromosome 9 deletions were identified as independent high risk factors (Table 2).

Considering that cytogenetic abnormalities are among the most important factors in predicting outcome, we analyzed a large series of patients with adult B-precursor ALL. We found that cytogenetic abnormalities involving chromosome arm 9p in adult B-precursor ALL define a group of patients with a remarkably short survival period after conventional treatment. The prevalence of abnormal 9p in this cohort of patients (8%) is similar to that in previous reports about chromosome 9p abnormalities in precursor B-cell ALL. These abnormalities are more frequent than in T-cell ALL.[12] The frequency of t(9;22), which defined a cytogenetic-molecular group with unfavorable outcome, was 33%, and our data are consistent with those reported by several groups in terms of poor outcome. Complex karyotype was associated with dismal prognosis, even though no further statistical analyses were performed owing to the low number of patients in this group. This result is in agreement with that previously reported by Moorman *et al*.[12]

Deletions in 9p have previously been regarded as representing an adverse risk factor in B-lineage, but not in T-cell ALL. Deletions in 9p21 have been studied in childhood ALL.[13,14] This locus encodes, among others, the p14ARF protein,[15,16] which binds to and inactivates HDM-2, which in turn targets p53 for degradation (the ARF-HDM2-P53 pathway). Thus, deletions in 9p21 result in inhibition of p53 protein.[17,18] Deletions/mutations of p53 protein are rare in ALL; the frequency of 17p deletions was 2% in our material. The protein encoded by *p16INK4a* activates the retinoblastoma tumor suppressor gene product, pRb. The protein encoded by *p15INK4b* is another tumor suppressor. It converts a hyperphosphorylated form of Rb protein into a hypophosphorylated form. Deletion of *p16INK4a* and *p15INK4b* occurs in almost all cases of childhood T-cell ALL and it might be associated with a favorable outcome in adult T-ALL.[19] The p53 protein and pRb are among the most important tumor suppressors involved in tumor progression and drug resistance.[20,21] In a recent study by Moorman *et al*.[12] del(9p) was indicated as being a good (but not an independent) prognostic marker of good prognosis in 71 (9%) patients in a population of patients aged between 15-65 years, with a mean age of 29 years. Sixteen (23%) of these patients had T-cell ALL. The number of patients with del(9p) who had undergone BMT was not defined. Similar results were reported by Mancini *et al*. among patients with del(9p)/p15-p16.[2] In contrast, Heerema *et al*.[4] reported that 9p aberrations were adverse risk factors in B-lineage ALL in a large pediatric study.

Patients with abnormal chromosome 9p in this study showed poor overall survival, with a mean survival period of only 13 months. When comparing this group with patients with normal karyotype, we found a highly significant difference. Abnormalities in 9p were found to represent an independent high risk factor and there was a similar unfavorable prognosis as regards t(9;22)/BCR-ABL. There are differences in patient populations between studies that may have had an impact on the overall results. We analyzed a pure B-precursor ALL population of adult patients with a high mean age (42 years, range 17-81), of whom only one had undergone BMT upfront.

In conclusion, in this study, abnormalities in chromosome arm 9p were identified as being adverse risk factors in B-precursor ALL. Owing to the divergent data regarding the prognostic impact of abnormalities in 9p, additional studies are warranted as regards both the relationship to immunophenotype and the role of intensified treatment by means of BMT. The challenge in future trials regarding del(9p) will also be to further extend the number of cytogenetic-molecular analysis techniques, such as RT-PCR and FISH.

Authorship and Disclosures

HN: data management, statistics, writing and planning the study; HH: provided information and clinical data on patients, helped in writing; TA: provided information and clinical data on patients, helped in writing; PB: provided information and clinical data on patients, helped in writing; MH: provided information and clinical data on patients, helped in writing; KAGK: provided information and clinical data on patients, helped in writing; Vladimir Lj Lazarevic, provided information and clinical data on patients, helped in writing; ML: provided information and clinical data on patients, helped in writing; BS: provided expert information and helped in writing; MÅ: provided information and clinical data on patients, helped in writing; HH: data management, statistics, writing and planning the study. The authors reported no potential conflicts of interest.

References

1. Hoelzer D, Gokbuget N. New approaches to acute lymphoblastic leukemia in adults: where do we go? Semin Oncol 2000;27:540-59.
2. Mancini M, Scappaticci D, Cimino G Nanni M, Derme V, Elia L, et al. A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. Blood 2005; 105:3434-41.
3. Heyman M, Grandér D, Bröndum-Nielsen K, Liu Y, Söderhäll S, Einhorn S. Deletions of the short arm of chromosome 9, including the interferon-α/-β genes, in acute lymphocytic leukemia. Studies on loss of heterozygosity, parental origin of deleted genes and prognosis. Int J Cancer 1993;54:748-53.
4. Heerema NA, Sather HN, Sensel MG Liu-Mares W, Lange BJ, Bostrom BC, et al. Association of chromosome arm 9p abnormalities with adverse risk in childhood acute lymphoblastic leukemia: A report from the Children's Cancer Group. Blood 1999;94:1537-44.
5. Martin TG, Gajewski JL. Allogeneic stem cell transplantation for acute lymphocytic leukemia in adults. Hematol Oncol Clin North Am 2001;15:97-120.
6. Thiebaut A, Vernant JP, Degos L, Huguet FR, Reiffers J, Sebban C, et al. Adult acute lymphocytic leukemia study testing chemotherapy and autologous and allogeneic transplantation. A follow-up report of the French protocol LALA 87. Hematol Oncol Clin North Am 2000;14:1353-66.
7. Gupta V, Yi QL, Brandwein J, Minden MD, Schuh AC, Wells RA, et al. The role of allogeneic bone marrow transplantation in adult patients below the age of 55 years with acute lymphoblastic leukemia in first complete remission: a donor vs no donor comparison. Bone Marrow Transplant 2004;33:397-404.
8. Schauer P, Arlin ZA, Mertelsmann R, Cirrincione C, Friedman A, Gee TS, et al. Treatment of acute lymphoblastic leukemia in adults: results of the L-10 and L-10M protocols. J Clin Oncol 1983;1:462-70.
9. Hallböök H, Hägglund H, Stockelberg D, Nilsson PG, Karlsson K, Björkholm M, et al. Autologous and allogeneic stem cell transplantation in adult ALL: the Swedish Adult ALL Group experience. Swedish Adult ALL Group. Bone Marrow Transplant 2005;35:1141-8.
10. Hallböök H, Simonsson B, Ahlgren T, Björkholm M, Carneskog J, Grimfors G, et al. High-dose cytarabine in upfront therapy for adult patients with acute lymphoblastic leukaemia. Br J Haematol 2002;118: 748-54.
11. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50: 163-70.
12. Moorman AV, Harrison CJ, Buck GA, Richards SM, Secker-Walker LM, Martineau M, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Adult Leukaemia Working Party, Medical Research Council/National Cancer Research Institute. Blood 2007;109:3189-97.
13. Kowalczyk J, Sandberg AA. A possible subgroup of ALL with 9p. Cancer Genet Cytogenet 1983;9: 383-5.
14. Diaz MO, Rubin CM, Harden A, Ziemin S, Larson RA, Le Beau MM, et al. Deletions of interferon genes in acute lymphoblastic leukemia. N Engl J Med 1990;322:77-82.
15. Siebert R, Willers CP, Schramm A, Fosså A, Dresen IM, Uppenkamp M, et al. Homozygous loss of the MTS1/p16 and MTS2/p15 genes in lymphoma and lymphoblastic leukaemia cell lines. Br J Haematol 1995;91:350-4.
16. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992;69:1237-45.
17. Bates S, Phillips AC, Clark PA, Stott F, Peters G, Ludwig RL, et al. p14ARF links the tumour suppressors RB and p53. Nature 1998;395: 124-5.
18. Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, et al. p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. Embo J 2001;20:1331-40.
19. Omura-Minamisawa M, Diccianni MB, Batova A, Chang RC, Bridgeman LJ, Yu J, et al. Universal inactivation of both p16 and p15 but not downstream components is an essential event in the pathogenesis of T-cell acute lymphoblastic leukemia. Clin Cancer Res 2000;6: 1219-28.
20. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 1993; 329:1318-27.
21. Schmitz NM, Hirt A, Aebi M, Leibundgut K. Limited redundancy in phosphorylation of retinoblastoma tumor suppressor protein by cyclin-dependent kinases in acute lymphoblastic leukemia. Am J Pathol 2006;169:1074-9.