Prognostic value of karyotypic analysis in children and adults with high-risk acute lymphoblastic leukemia included in the PETHEMA ALL-93 trial

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Background and Objectives. Cytogenetic analysis is one of the most reliable prognostic factors in acute lymphoblastic leukemia. The objective of this study was to analyze the prognostic value of cytogenetic analysis in children and adults with high-risk acute lymphoblastic leukemia (HR-ALL) included in a prospective multicenter trial.

Design and Methods. One hundred and thirty patients (44 children and 86 adults) with HR-ALL included in the PETHEMA ALL-93 trial had an adequate cytogenetic study after review. Cytogenetic subgroups were established according to the cancer and acute leukemia group B criteria (unfavorable: 11q23, t(9;22), -7 and +8; normal; miscellaneous: the remaining chromosome abnormalities) and their main clinicobiological features were compared. Univariable and multivariable analyses for complete remission (CR) attainment, event-free survival (EFS) and overall survival (OS) were performed.

Results. The mean±SD age was 26±14 years. Two were infants (<1 year), 42 were children and 86 adults (19-50 years). The cytogenetic study was normal in 44 (34%) cases. The most frequent chromosomal rearrangement was t(9;22)(q34;q11) (34 cases, 26%, 30 adults), followed by 11q23 (12 cases, 9% -8 children-, including t(4;11)(q21;q23) in 8, 7 children). Patients with t(9;22) were older than the remaining cases, whereas those with 11q23 rearrangements were younger and had higher WBC counts. Multivariable analyses showed two associated factors in adults with a lower frequency of CR and a shorter EFS and OS: t(9;22) and slow response to therapy (assessed by a percentage of blast cells higher than 10% in bone marrow study on day 14). For children with very high-risk ALL, only slow response to therapy (assessed by the presence of blast cells in peripheral blood on day 8) was associated with a negative impact on CR, EFS and OS.

Interpretation and Conclusions. In adult patients with high-risk acute lymphoblastic leukemia included in the PETHEMA ALL-93 protocol, cytogenetic analysis at diagnosis is a useful independent prognostic marker. The poorest prognosis for patients with t(9;22) justifies the development of specific treatments for these patients. In this small subgroup of children with very high-risk ALL no cytogenetic characteristics was found to influence the results of therapy, slow response to therapy being the only prognostic factor.

Key words: acute lymphoblastic leukemia, children, adults, high-risk, cytogenetics, t(9;22).

Karyotypic abnormalities are one of the most important prognostic factors for children and adults with acute lymphoblastic leukemia (ALL).[1,2] Several alterations, e.g.: t(9;22)(q34;q11), t(4;11)(q21;q23), are typically associated with poor prognosis, and together with age, white blood cell count and slow response to therapy, define the group of patients with high-risk ALL (HR-ALL).[3,4] Intensive induction and post-remission therapy, including stem cell transplantation (SCT), have improved the outcome of patients with HR-ALL.[5-11] From the results of large multicenter trials cytogenetic analysis retains its prognostic significance

in HR-ALL patients.[12,13] However, some of these studies are based on patients included in different consecutive trials with different treatment intensities, a feature with possible influence on the results.[13,14]

The aim of this study was to analyze the influence of cytogenetics on a series of children and adults with HR-ALL included in a prospective multicenter randomized trial (ALL-93).

Design and Methods

Patients and diagnostic criteria

From June 1993 to June 2000, 203 previously untreated HR-ALL patients from 32 Spanish centers were prospectively included in the PETHEMA (*Programa para el Estudio y Tratamiento de las Hemopatías Malignas, Spanish Society of Hematology*) ALL-93 protocol. The diagnosis of ALL was made according to morphologic (FAB classification,[15,16] immunologic and cytogenetic criteria. Bone marrow and peripheral blood specimens were stained by standard techniques, including May-Grünwald-Giemsa, periodic acid Schiff reagent, myeloperoxidase, acid phosphatase and naphthol ASD acetate esterase. Immunologic study was performed by flow cytometry using a panel of monoclonal antibodies labeled with fluorescein isothiocyanate or phycoerythrin reactive with lymphoid and myeloid antigens (CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD22, CD13, CD14, CD33, CD34, anti-myeloperoxidase and HLA-DR). In addition, Tdt, sIg and intracytoplasmic μ chain were investigated by immunofluorescence techniques. The criterion for marker positivity was expression of the antigen by at least 20% of the leukemic blast population. Four immunologic subtypes of ALL were considered: *early pre-B* (CD19$^+$, CD 10-, intracytoplasmic μ chain [μIC] -), *common* (CD19$^+$, CD202$^{+/-}$, CD10$^+$, μIC-), *pre-B* (CD19$^+$, CD202$^{+/-}$, CD102$^{+/-}$, μIC$^+$), and *T-ALL* (CD7$^+$, cCD3$^+$, CD52$^{+/-}$, CD2$^{+/-}$, CD12$^{+/-}$). The presence of myeloid (My) antigens was also evaluated. Myeloid antigen expression was defined as CD13, CD14 and/or CD33 positivity co-expressed with eiher T- or B-lineage antigens on blast cells. Patients expressing anti-myeloperoxidase (MPO) were excluded from the study.

Inclusion criteria for children (age ≤18 years) and adults (age 19-50 years) are shown in Table 1. Patients with prior or concomitant malignancy, previous treatment for ALL, ALL-L3 (Burkitt's-type ALL), uncontrolled or severe cardiovascular disease, pre-existing liver disease or psychiatric disease were not eligible for this study. Patients provided informed consent before entering the study.

Table 1. PETHEMA ALL-93. Inclusion criteria.

Children (age ≤18 year). One or more of the following:
Age < 1 year
WBC count >100×10^9/L and T-cell phenotype
WBC count >200×10^9/L
t(9;22)
t(4;11) or other 11q23 rearrangements
Adults (age > 18 year). One or more of the following:
Age 30-50 year
WBC count >25×10^9/L
t(9;22)
t(4;11) or other 11q23 rearrangements

Cytogenetic analyses

Chromosomal analyses of bone marrow were performed at diagnosis in institutional laboratories and the results were reviewed centrally by two of the authors (IG and J-MHR). Specimens were processed using direct methods and unstimulated short-term (24 and 48-hour) cultures. G-banding was performed. The definitions of hyperdiploid or hypodiploid karyotypes were only based on standard cytogenetic investigations. DNA index by flow cytometry was not measured. A minimum of 20 bone marrow metaphase cells were required in each patient designated as having a normal karyotype. The criteria of the International System for Human Cytogenetic Nomenclature were employed to describe a cytogenetic clone and for the karyotype description.[17]

Treatment and criteria for response

The treatment of ALL is shown in Table 2.[18] Briefly, induction treatment included a 5-week conventional therapy with vincristine, prednisone, L-asparaginase, daunorubicin and cyclophosphamide. In all patients who achieved complete remission (CR) HLA typing was performed. Following CR patients received three monthly cycles of early intensification chemotherapy including cytotoxic drugs active against ALL at intermediate or high-doses (Table 2). Central nervous system (CNS) prophylaxis consisted of intrathecal chemotherapy with methotrexate, cytosine arabinoside and hydrocortisone beginning in the induction phase and given throughout the first year of treatment (12 doses), in addition to high-dose intravenous methotrexate given in the first two cycles of early intensification therapy. Hematopoietic growth factors (G-CSF) were used after each intensification cycle. Patients not achieving CR received the first intensification cycle and if no CR was then

achieved they were excluded from the protocol. Patients with an HLA-identical sibling were assigned to allogeneic SCT whereas the remaining patients were randomized to receive autologous SCT or the same three cycles of chemotherapy used in the early intensification phase followed by conventional maintenance treatment (daily oral mercaptopurine and weekly intramuscular methotrexate) until two years after CR achievement (Table 2). Randomization was performed after CR, when the results of the HLA study were known. No additional cytotoxic or immunomodulatory treatment was given to patients submitted to allogeneic or autologous SCT. The use of hospitalization, the prophylaxis and management of infections and the transfusion policy were not prescribed by protocol and were performed according to the specific protocols of each participating hospital.

Early death (ED) was considered as a death occurring before response to therapy could be established. Patients were considered to be in CR when all extramedullary disease had resolved, the neutrophil count was higher than 1.5×10^9/L, the platelet count was greater than 100×10^9/L, and there was normal bone marrow cellularity (>25%) with trilineage hematopoiesis and less than 5% blast cells. According to the criteria from previous studies of the PETHEMA Group,[19] two patterns of response were considered: *slow*, defined as the presence of peripheral blood blast cells (PBBC) on the 8th day of therapy or >10% blast cells in the bone marrow aspirate performed on day 14 of treatment, and *fast*, defined as the absence of PBBC on the 8th day and ≤10% BM blast cells (BMBC) on day 14. Relapse was defined by the reappearance of more than 5% leukemic cells in the bone marrow aspirates or extramedullary leukemia in patients with a previously documented CR. Event-free survival (EFS) was defined to be the time between the diagnosis and failure of therapy, relapse (bone marrow or extramedullary), death from any cause or last follow-up alive in first CR; the events were early death, resistance to therapy, relapse and death. Overall survival (OS) was measured from the time of entry into the protocol to the time of death or last follow-up. The analysis of EFS and OS probabilities according to the therapeutic option (allogeneic SCT, autologous SCT and intensification and maintenance chemotherapy) was made by intention-to-treat. All relapse and survival data were updated on July 30, 2001 and all follow-up data were censored at this point.

Table 2. PETHEMA ALL-93: chemotherapy schedule.

Phase	*Week no.*	*Route*	*Dose*	*Days*
Induction				
Vincristine	1-4	IV	2 mg	1,8,15,22
Daunorubicin	1-4	IV	30 mg/m²	1,8,15,22
Prednisone	1-4	IV/PO	60 mg/m²	1-28
	5	IV/PO	30 mg/m²	29-33
	5-6	IV/PO	15 mg/m²	34-38
L-asparaginase	3,4	IV	10,000 IU/m²	16-20, 23-27
Cyclophosphamide	5	IV	1,000 mg/m²	36
CNS prophylaxis				
Methotrexate	1,4,7,11,15 21,25,29,33, 37,41,45	IT	15 mg	1,28,49,77,105, 175,203,231,259, 287,315
Cytarabine	Idem	IT	30 mg	Idem
Hydrocortisone	Idem	IT	20 mg	Idem
Early intensification-1				
Vincristine	7-8	IV	2 mg	1,8
Dexamethasone	7-8	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Methotrexate	7	IV	3 g/m²	1
Cytarabine	7	IV	2 g/m²/12h	5
L-asparaginase	7	IV/IM	25,000 IU/m²	5
Mercaptopurine	7	PO	100 mg/m²	1-5
Early intensification-2				
Vincristine	11-12	IV	2 mg	1,8
Dexamethasone	11-12	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Methotrexate	11	IV	3 g/m²	1
Cyclophosphamide	11	IV	150 mg/m²	1-5
L-asparaginase	11	IV/IM	25,000 IU/m²	5
Mitoxantrone	11	IV	12 mg/m²	5
Early intensification-3				
Dexamethasone	15-16	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Cytarabine	15	IV	2 g/m²/12h	1-2
Teniposide	15	IV	150 mg/m²	3-4
L-asparaginase	11	IV/IM	25,000 IU/m²	5
Delayed intensification-1#				
Vincristine	19-20	IV	2 mg	1,8
Dexamethasone	19-20	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Methotrexate	19	IV	3 g/m²	1
Cytarabine	19	IV	2 g/m²/12h	5
L-asparaginase	19	IV/IM	25,000 IU/m²	5
Mercaptopurine	19	PO	100 mg/m²	1-5
Delayed intensification-2#				
Vincristine	23-24	IV	2 mg	1,8
Dexamethasone	23-24	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Methotrexate	23	IV	3 g/m²	1
Cyclophosphamide	23	IV	150 mg/m²	1-5
L-asparaginase	23	IV/IM	25,000 IU/m²	5
Mitoxantrone	23	IV	12 mg/m²	5
Delayed intensification-3#				
Dexamethasone	27-28	IV/PO	20 mg/m²	1-5
		IV/PO	10 mg/m²	6
		IV/PO	5 mg/m²	7
		IV/PO	2.5 mg/m²	8
Cytarabine	27	IV	2 g/m²/12h	1-2
Teniposide	27	IV	150 mg/m²	3-4
L-asparaginase	27	IV/IM	25,000 IU/m²	5
Maintenance#				
Mercaptopurine	31-104	PO	60 mg/m²	Daily
Methotrexate	31-104	IM	15 mg/m²	Weekly

#Only for patients randomized to receive chemotherapy.

Statistical analysis

A descriptive study of the main clinical and hematologic variables of ALL patients included in the various karyotypic subgroups was performed. *p* values for comparisons of continuous variables between groups of patients were based on the Wilcoxon rank sum test. *p* values for dichotomous variables were based on Pearson's χ^2 test or Fisher's exact test when appropriate. EFS and OS curves were plotted by the Kaplan and Meier method[20] and were compared by the log-rank test.[21] The statistically significant variables ($p<0.05$) or those with borderline statistical significance ($0.05<p<0.1$) identified in univariable studies were included in multivariable analyses. A logistic regression model was used to identify predictive factors for CR attainment, whereas multivariable analyses for EFS and OS were performed using the Cox proportional hazards regression model.[22] In multivariable analyses logarithmic transformation of the WBC count was performed. Ninety percent confidence intervals for probabilities and median survival times were calculated.[23] The significance level was fixed at $p = 0.05$ and all *p* values were two-sided unless otherwise stated. Statistical analyses were carried out using the SPSS (Statistical Package for Social Sciences) package version 9.0 for Windows.

Results

Patient characteristics and response to therapy

A total of 203 patients with HR-ALL were included in the PETHEMA ALL-93 protocol. No cytogenetic sample was available for 26, and after review cytogenetic analyses were considered inadequate in 47 (no mitoses: 29 cases, normal karyotype but fewer than 20 mitoses: 18 cases). Thus, 130 out of 203 (64%) cases were eligible for this study. The mean (SD) age for this group was 26(14) year, 44 were children (2 were infants) and 86 adults. ED occurred in 4 cases (3%)(1 child and 3 adults), refractory ALL was observed in 19 (15%)(4 children and 15 adults) and CR was attained in 106 (82%) patients (38 children and 68 adults). In 7 out of 106 patients (5%)(3 children and 4 adults), CR was attained after the addition of cycle 1. Bone marrow blast cells >10% on day 14 were observed in 51 (18 children and 33 adults) out of 126 (40%) evaluable patients. With a median follow-up time for the whole series of 17 months (range 1-78) in living patients the median (95% CI) times for EFS and OS were 11 (8-17) and 21 (13-29) months, respectively, with projected 4-year EFS and OS (95%CI) probabilities of 23% (11-35) and 27% (11-38). For the 73 patients not eligible for this report, the OS (median 28 months, 95%CI 21-35), and EFS (median 13 months, 95%CI 7-19) curves were not significantly different from that of evaluable patients. For children these probabilities were 23% (5-41) and 35% (17-53) respectively, being 20% (6-38) and 21% (3-39), respectively for adults. The median (range) time between CR and SCT was 151 (69-201) days for allogeneic and 159 (85-270) days for autologous transplant recipients. By intention-to treat-analysis, there are so far no differences in either EFS or OS between children or adult patients receiving allogeneic SCT (n= 43), autologous SCT (n= 29) or intensification chemotherapy (n=28).

Table 3. Frequency of recurring chromosomal abnormalities in the whole series as well as in children and in adults.

Abnormality	*Total No. (Ch/Ad)*	*Associated abnormalities (Ch/Ad)*				
		+8	*–7*	*11q23*	*Other*	*None*
No abnormality[1]	44 (18/26)	–	–	–	–	18/26
t(9;22)(q34;q11)	34 (4/30)	0/3	0	0/2	1/5	3/20[2]
t(4;11)(q21;q23)	9 (7/2)	0	0	–	2/0	5/2
Other 11q23 abnorm.	3 (1/2)	0	0	–	0	1/2
+8	2[3] (2/0)	–	0	–	1/0	1/0
-7	5 (2/3)	0	–	0	2/0	1/2
Hyperdiploidy >50[8]	8[4] (3/5)	1/0	–	–	0/1	2/4
Hypodiploidy not -7[8]	9 (2/7)	–	–	–	0/5	2/2
6q or 6p deletions	5 (1/4)	0	0	1[5]/0	0/3	0/1
Other translocations	6[6] (3/3)	–	–	–	1/2	2/1
Other deletions	5[7] (1/4)	–	–	–	1/3	0/1

Ch: children, Ad: adults. [1]Includes one patient with constitutional 47,XX +21 and one case with 47,XXX. [2]Includes two patients 46,XX and 46,XY with BCR/ABL+ by molecular methods. [3]Cases with +8 associated with t(9;22) are not included in this category. [4]Does not include a case 53,XX with +8 and t(9;22). [5]A case involving 11q in which the breakpoint could not be defined. Not included in 11q23 category. [6]Includes one case with t(1;19), one case with t(1;9), one case with t(6;14) and two cases with more than four chromosomes involved. [7]Includes one case with del(15) and four cases with abnormalities involving several chromosomes. [8]The definitions of hyperdiploid and hypodiploid karyotypes were based only on standard cytogenetic investigations.

Cytogenetic subgroups

Cytogenetic study was normal in 44 (34%) (18 children and 26 adults) of the evaluable cases. The frequencies of specific chromosomal abnormalities (following the Cancer and Acute Leukemia Group B criteria),[12] in the whole series as well as in children and in adults separately are summarized in Table 3. It is of note that in the ALL-93 protocol the frequencies of patients with no metaphases or normal karyotype but less than 20 metaphases were 16% and 10%, respectively. These frequencies are not significantly different from those observed in con-

Table 4. Pre-treatment characteristics of infant and childhood patients by cytogenetic risk group.

Characteristics	Risk group –7/+8	t(9;22)	11q23	Standard	Total
No. of patients	4	4	8	28	44
Age (yr)*					
Median	14	11	3	16	14
(range)	(7-18)	(7-17)	(0-18)	(1-18)	(0-18)
Infants			2		
Sex					
Males	2	3	4	14	23
Females	2	1	4	14	21
Mediastinal mass*	2	0	1	6	9
Hepatomegaly	1	1	4	13	19
Splenomegaly	1	2	7	16	26
Lymphadenopathy	1	2	1	17	21
Testicular infiltration	0	0	0	1	1
CNS infiltration	1	0	0	2	3
WBC count ($\times 10^9$/L)*					
Median	42	68	317	32	44
Range	(1-410)	(8-822)	(12-400)	(2-620)	(1-822)
> 50.0	1	2	7	11	21
Platelet count ($\times 10^9$/L)					
Median	84	53	41	51	53
Range	(56-135)	(31-83)	(19-335)	(4-375)	(3-375)
Hemoglobin (g/L)					
Median	110	102	86	95	99
Range	(57-120)	(66-114)	(63-109)	(35-154)	(35-154)
Immunophenotype*					
Early-preB	0	0	7	2	7
Early-PreB/My+	1	0	1	3	4
Common	1	2	0	7	10
Common/My+	0	2	0	4	7
T	1	0	0	6	8
T/My+	1	0	0	6	8

*Standard karyotype: patients with normal, hyperdiploid, hypodiploid and miscellaneous karyotypes. MY+: with myeloid markers. *p < 0.05 between categories.*

Table 5. Pre-treatment characteristics of adult patients by cytogenetic risk group.

Characteristics	Risk Group -7/+8	t(9;22)	11q23	Standard	Total
No. of patients	3	30	4	49	86
Age (yr.) *					
Median	20	38	26	32	33
(range)	(19-33)	(19-50)	(22-50)	(19-50)	(19-50)
Sex					
Males	2	15	2	31	50
Females	1	15	2	18	36
Mediastinal mass	1	0	0	4	5
Hepatomegaly	1	3	0	6	10
Splenomegaly	1	12	0	12	25
Lymphadenopathy	2	3	2	19	25
Testicular infiltration	0	0	0	2	2
CNS infiltration	0	0	0	2	2
WBC count ($\times 10^9$/L)*					
Median	26	17.3	142	17	1
Range	(7-46)	(2-300)	(6.7-288)	(1-204)	(1-300)
>50.0	0	9	2	13	24
Platelet count ($\times 10^9$/L)					
Median	77	32	92	53	37
Range	(5-83)	(3-162)	(12-210)	(4-260)	(3-260)
Hemoglobin (g/L)					
Median	110	98	119	103	100
Range	(90-133)	(50-144	(72-139)	(45-152)	(45-152)
Immunophenotype*					
Early-preB	0	0	1	8	10
Early-PreB/My+	1	0	2	7	10
Common	0	15	1	10	24
Common/My+	1	15	0	9	26
T	0	0	0	10	10
T/My+	1	0	0	5	6

*Standard karyotype: patients with normal, hyperdiploid, hypodiploid and miscellaneous karyotypes. MY+: with myeloid markers. *p<0.05 between categories.*

temporary PETHEMA protocols for low-risk ALL patients (10% and 14%, respectively) and intermediate-risk ALL patients (12% and 13%, respectively). Since there were no differences in the response to therapy between HR-ALL patients with normal, hyperdiploid, hypodiploid and miscellaneous karyotypes, the patients were pooled as a standard group for further comparisons. The specific survival curves for patients with hypodiploidy and hyperdiploidy were as follows; for hypodiploid cases the median (95%CI) OS was 34 (0-70) months, vs. 21(10-31) months for non-hypodiploid cases (log-rank = 0.55, p=0.46); for hyperdiploid patients the median OS was 20.9 (0.8-40.9) months vs. 20.8 (12.0-29.5) months for non-hyperdiploid cases (log-rank = 0.02, p=0.88). The comparison of the main pre-treatment characteristics of the 130 ALL patients grouped by cytogenetic characteristics showed that patients with t(9;22) were older while patients with 11q23 rearrangements were younger than those included in the standard group. For 11q23 patients the mean (SD) age was 13.4 (15.3) years vs. 27.4 (12.8) for standard group patients (t=3.5, p=0.001). In addition, the highest WBC counts were observed in patients carrying 11q23 rearrangements. The mean (SD) WBC count was 239(152)$\times 10^9$/L for 11q23 patients vs. 71(122)$\times 10^9$/L for standard patients (t = -4.4, p<0.001). No other relevant clinical differences

Table 6. Univariable analysis of prognostic factors for complete remission attainment.

Variable	*Category*	*N (Ch/Ad) N (Ch/Ad)*	*CR N (Ch/Ad)*	*No CR (Ch/Ad)*	*p*
Age (yr)	1-18	44	40	4	0.108
	>18	86	68	18	
WBC count (×10⁹/L)	<50	85 (23/62)	70 (21/49)	15 (2/13)	NS*
	≥50	45 (21/24)	37 (18/19)	8 (3/5)	
Mediastinal mass	yes	14 (9/5)	14 (9/5)	0	NS
	no	116 (35/81)	93 (30/63)	23 (5/18)	
FAB category	L1	48 (27/21)	43 (25/18)	5 (2/3)	NS
	L2	82 (17/65)	64 (14/50)	18 (3/15)	
Immunophenotype T lineage					
T/TMy		31 (14/17)	29(14/15)	2 (0/2)	NS
B lineage/BMy		98 (29/69)	77 (24/53)	21(5/16)	
Early pre-B		32 (13/19)	27 (11/16)	5 (2/3)	
Common+pre-B		66 (16/50)	50 (13/37)	16 (3/13)	
No My		71 (25/46)	57 (21/36)	14 (4/10)	
My		59 (19/40)	50 (18/32)	9 (1/8)	
Cytogenetic group**					
Standard		77 (28/49)	68 (25/43)	9 (3/6)	NS/0.009°
-7/+8		7 (4/3)	5 (4/3)	0	
t(9;22)		34 (4/30)	22 (3/19)	12 (1/11)	
11q23		12 (8/4)	10 (7/3)	2 (1/1)	
PB blast cells/d+8					
No		100 (34/66)	87 (33/54)	13 (1/12)	0.003/NS
Yes		24 (8/16)	16 (5/11)	8 (3/5)	
Blasts in BM/d+14					
≤10%		75 (24/51)	71 (24/47)	4(0/4)	0.015/<0.001
>10%		51 (18/33)	34 (14/20)	17 (4/13)	

*Ch: children; Ad: adults; CR: complete remission. FAB: French-American-British classification; N: number of patients; PB: peripheral blood; BM: bone marrow; MY: myeloid markers. *No differences were observed when other cut-off points were analyzed. °t(9;22) vs remaining.**Standard group consists of patients with normal cytogenetic analysis or abnormalities other than t(9;22), t(4;11), -7, or +8.*

Table 7. Univariable analysis of prognostic variables for event-free survival (EFS) and overall survival (OS) in children with acute lymphoblastic leukemia.

			EFS			*OS*		
Variable	*Cat.*	*N*	*Median months (95% CI)*	*Percent in CCR 2 yr (95% CI)*	*p*	*Median months (95% CI)*	*Percent alive (95% CI)*	*p*
WBC count (×10⁹/L)	<40	21	35 (0-77)	49 (18-77)	0.06	– –	65 (43-87)	0.01
	>40	23	9 (4-14)	18 (1-38)		12 (11-13)	25 (7-45)	
Mediastinal mass								
	yes	9	9 (0-23)	22 (0-48)	0.42	12 (10-14)	25 (0-66)	0.18
	no	35	11 (6-17)	38 (14-60)		26 (13-39)	54 (44-64)	
FAB subtype	L1	27	11 (6-16)	31 (5-47)	0.85	26 (6-47)	46 (21-70)	0.18
	L2	17	12 (6-17)	32 (20-46)		24 (1-48)	43 (19-61)	
Immunophenotype								
	T/TMy	14	7 (2-12)	15 (0-40)	0 .07	12 (10-13)	28 (18-54)	0.03
	B/BMy	30	13 (0-36)	44 (14-69)		33 –	58 (38-72)	
	No My	25	10 (8-13)	20 (0-62)	0.80	34 (1-67)	45 (11-71)	0.84
	My	19	9 (4-15)	30 (7-54)		24 (7-42)	47 (22-65)	
Cytogenetic group*								
	Standard	28	9 (6-13)	31 (6-53)	0.24	24 (4-45)	48 (30-65)	0.76
	-7/+8	4	11 (1-21)	33 (0-85)		– (–)	– (–)	
	t(9;22)	4	3 (0-11)	12 (0-66)		– (–)	– (–)	
	11q23	8	13 (–)	50 (0-100)		22 (7-38)	40 (0-80)	
Blasts in PB on day +8								
	No	34	11 (5-17)	40 (18-59)	0.02	26 (12-40)	54 (36-75)	0.05
	Yes	8	3 (0-8)	12 (0-34)		7 (3-11)	13 (0-51)	

*Abbreviations: CI, confidence interval; FAB, French-American-British classification; N, number of patients; PB, peripheral blood. No differences in EFS or OS were observed between sexes, and early-pre-B vs common+pre-B phenotypes. *Standard group consists of patients with normal cytogenetic analysis or abnormalities other than t(9;22), t(4;11), -7, or +8.*

were observed. Table 4 shows the comparison of the main pre-treatment characteristics of the 44 children grouped by cytogenetic subtypes. Children with 11q23 rearrangements were younger, had higher WBC counts and more frequently carried early pre-B phenotype than the remaining cases. Table 5 shows the relationship of initial characteristics and cytogenetic subgroups in the 86 adult patients from the series. Patients with 11q23 had higher WBC counts than the remaining, and cases with t(9;22) more frequently showed a common phenotype than those from the remaining subgroups.

The most frequent cytogenetic rearrangement was t(9;22) (34 cases, 26%, 4 children and 30 adults). Within this group of patients, additional chromosomal abnormalities were present in 11 out of the 34 (33%) cases (Table 3). No differences in response to therapy and survival were found between Ph+ ALL cases with (n=11, 1 children and 10 adults) and without (n=23) additional cytogenetic abnormalities (CR 73% vs. 61%, median EFS 8 vs. 5.6 months, median OS 15 vs.10 months, respectively).

Table 8. Univariable analysis of prognostic variables for event-free survival (EFS) and overall survival (OS) in adults with acute lymphoblastic leukemia.

			EFS			OS		
Variable	*Category*	*N*	*Median months (95%CI)*	*Percent in CCR 2 yr (95%CI)*	*p*	*Median months (95%CI)*	*Percent alive 2 yr (95%CI)*	*p*
WBC count ($\times 10^9$/L)	<50	62	17 (9-25)	24 (11-41)	0.48	19 (12-26)	37 (21-51)	0.099
	>50	24	11 (4-17)	38 (19-58)		25 (14-37)	49 (27-74)	
Mediastinal mass								
	yes	5	12 (6-18)	28 (13-40)	0.23	– (–)	75 31-100)	0.13
	no	81	23 (–)	50 (0-100)		19 (13-25)	41 (29-52)	
FAB subtype	L1	21	19 (3-34)	31 (6-57)	0.47	26 (14-39)	52 (24-73)	0.19
	L2	65	12 (5-18)	30 (2-42)		19 (10-27)	39 (23-53)	
Immunophenotype								
	T/TMy	17	– (–)	52 (27-78)	0.02	– (–)	63 (38-85)	0.02
	B/BMy	69	11 (7-15)	21 (10-37)		16 (8-23)	35 (21-49)	
	No My	46	12 (9-15)	32 (17-51)	0.96	21 (13-25)	43 (26-59)	0.97
	My	40	17 (4-30)	27 (11-42)		19 (9-29)	41 (12-55)	
Cytogenetic group*								
	Standard	49	23 (14-33)	43 (21-59)	0.003	28 (14-43)	54 (38-71)	0.01
	-7/+8	3	23 (0-56)	33 (0-83)		– (–)	– (–)	
	t(9;22)	30	6 (0-12)	4 (0-19)		13 (8-17)	11 (0-23)	
	11q23	4	3 (0-26)	25 (0-67)		35 (0-76)	49 (0-100)	
Blasts in BM on day +14								
	≤10%	51	17 (9-26)	40 (21-56)	0.004	26 (11-42)	51 (34-63)	0.003
	>10%	33	8 (2-14)	0 (–)		13 (6-19)	24 (6-48)	

Abbreviations: CI, confidence interval; FAB, French-American-British classification; N, number of patients; BM, bone marrow. No differences in EFS or OS were observed between sexes, and early-pre-B vs common+pre-B phenotypes Standard group consists of patients with normal cytogenetic analysis or abnormalities other than t(9;22), t(4;11), -7, or +8.

Table 9. Multivariable analyses of prognostic factors for event-free survival (EFS) and overall survival (OS) in children and in adults from the series.

	Stepwise Logistic Regression for CR			
Variables	*β*	*Risk (OR)*	*95%CI of OR*	*p(Wald)*
Children				
PB blasts on day +8	– 2.99	0.05	0.004-0.58	0.017
Adults				
BM blasts on day +14	– 2.04	0.13	0.04-0.47	0.002
t(9;22)	– 1.36	0.26	0.08-0.86	028

	Stepwise Cox Regression for EFS			
Variables	*β*	*Risk (OR)*	*95%CI of OR*	*p(Wald)*
Children				
PB blasts on day +8	1.02	2.77	1.32-6.78	0.026
Adults				
t(9;22)	1.19	3.29	1.74-6.19	<.001
BM blasts on day +14	0.94	2.55	1.36-4.78	0.004
11q23 components	0.69	2.00	0.58-6.96	NS
-7/+8	0.03	1.03	0.24-4.41	NS

	Stepwise Cox Regression for OS			
Variables	*β*	*Risk (OR)*	*95%CI of OR*	*p(Wald)*
Children				
PB blasts on day +8	1.2	3.44	1.38-8.59	0.008
Adults				
t(9;22)	0.88	2.41	1.27-4.55	0.007
BM blasts on day +14	0.84	2.32	1.22-4.44	0.011
11q23 components	0.09	1.10	0.25-4.85	NS
-7/+8	– 0.65	0.52	0.07-3.96	NS

BM: bone marrow; PB: peripheral blood, OR odds ratio.

The second most frequent chromosomal abnormality involved the 11q23 region (12 cases, 9%, 2 infants, 6 children and 4 adults). Most of these cases (9 out of the 12) had t(4;11), including the two cases of infant ALL. This latter translocation was associated with additional chromosomal abnormalities in only two cases (both children). Although the number of patients is too small to reach definitive conclusions, no differences in prognosis were observed between patients with t(4;11) and those with other 11q23 rearrangements.

Univariable and multivariable analyses of response to therapy and survival

Table 6 depicts the results of the univariable analysis for CR attainment in the whole series as well as separately in children and in adults. Although children had a higher CR rate than adults (90% vs. 79%), the difference did not reach statistical significance. For children the only parameter associated with a lower probability of CR was slow response to treatment, measured either as the presence of blast cells on a peripheral blood smear on the 8th day of induction therapy or as >10% blast cells in bone marrow study on day 14. In adults, t(9;22) and slow response to therapy (>10% blast cells in bone marrow on day 14) were associated with a lower probability of CR. Tables 7 and 8 show the results of univariable analyses for EFS

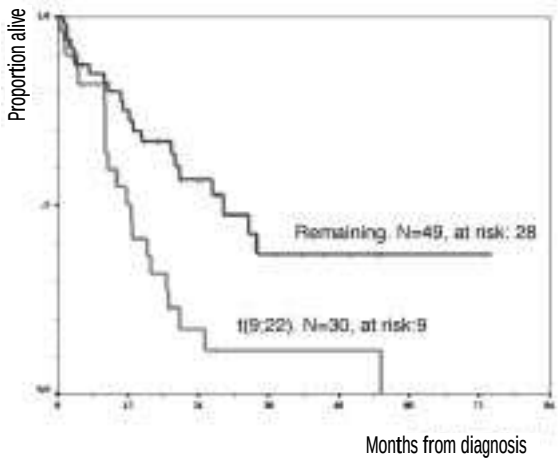

Figure 1. Actuarial curves of overall survival for adults according to the presence of t(9;22).

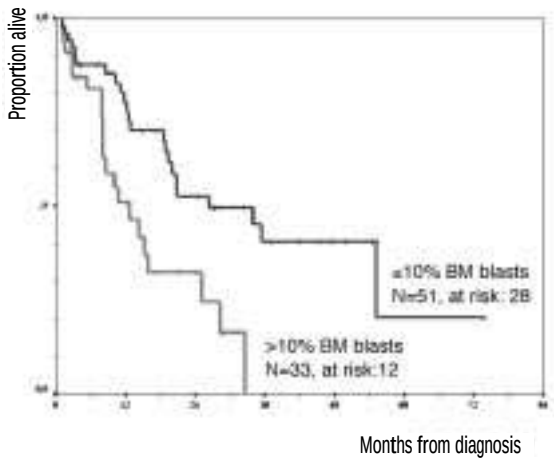

Figure 2. Actuarial curves of overall survival for adults according to the rate of response to induction therapy assessed by the percentage of bone marrow (BM) blast cells on day 14.

and OS for children and adults, respectively. For children, the main parameters associated with a lower EFS and OS were WBC count (cut-off point 40×10^9/L) and slow response to therapy (assessed by the presence of blast cells on day 8 of induction therapy). For adults, the main unfavorable prognostic factors for both EFS and OS were t(9;22) and the presence of >10% BMBC on day 14 of induction therapy. Adult patients with t(9;22) had the worst prognosis with an EFS significantly lower than that of the standard group (median 6 vs. 23 months, p=0.003) and OS (median 13 vs. 28 months, p=0.01).

Table 9 shows the results of multivariable analyses in children and in adults. Two factors in adults were associated with a lower frequency of CR and a shorter EFS and OS: t(9;22) and slow response to therapy (assessed by a percentage of blast cells higher than 10% in bone marrow study on day 14) (Figures 1 and 2). For children with very high-risk ALL, only slow response to therapy (assessed by the presence of blast cells in peripheral blood on day 8) was associated with a negative impact on CR, EFS and OS (Figure 3).

Discussion

The present study reports the prognostic factors identified in a series of high-risk ALL patients, defined by criteria of age, WBC count and karyotype, who were uniformly treated according to the PETHEMA ALL-93 protocol. This trial compares three post-remission and consolidation therapies: allogeneic SCT, autologous SCT and intensification plus maintenance chemotherapy. Preliminary results indicate that by intention-to-treat analysis, there are so far no differences in either EFS or OS between these three therapeutic options. This matter has been investigated in several studies[24-30] and is currently being evaluated in others.[31,32] Our results show that the two most relevant prognostic factors for adults with high-risk ALL are cytogenetics (t9;22) and BM blast cells at day +14 (>10%). For children with very high-risk ALL, only slow response to treatment was associated with a worse prognosis.

One limitation of this study is that only standard cytogenetic studies were performed. For this reason the true incidence of high-risk cytogenetic features in this study could not be evaluated. For example, recent data have shown that cytogenetic studies may fail to detect 11q23 rearrangements. In addition, the samples were processed in several reference laboratories, although a central review of the cytogenetic results was performed. The low number of patients, especially children, could have had an influence on the results. In spite of these limitations, cytogenetic results were similar to those reported by other groups, with a frequency of abnormal karyotypes (66%) and a distribution of numerical and structural chromosomal lesions within the range of those reported from other multicenter

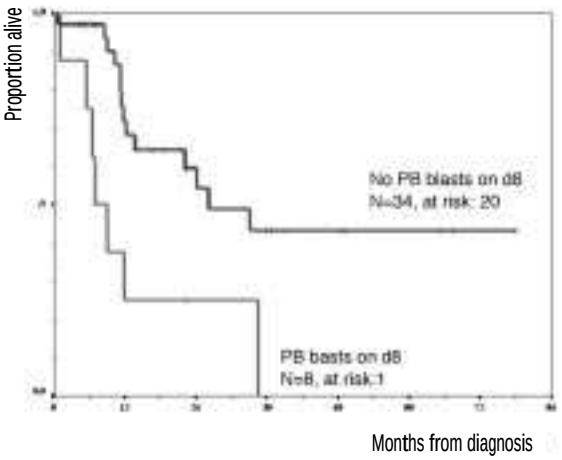

Figure 3. Actuarial curves of overall survival for children according to the rate of response to induction therapy assessed by the presence of peripheral blood (PB) blast cells on day 8.

studies.[12-14,33,34] The presence of 36% of cases not evaluable for this study is a matter of concern. Several efforts should be made to improve the results of cytogenetic studies in multicenter trials, i.e., by the systematic and centralized use of complementary techniques such as fluorescent *in situ* hybridization (FISH) and molecular biology. FISH techniques should include the use of specific probes to improve the detection of high-risk-defining abnormalities such as t(9;22), and centromeric probes for chromosomes 7 and 8. Molecular biology techniques should be systematically used to detect BCR/ABL and MLL rearrangements.

Recently, the prognostic value of several cytogenetic abnormalities in childhood or adult ALL has been reassessed in the setting of multicenter contemporary trials,[12-14, 34-39] and some chromosomal changes have lost their prognostic significance.[40] However, the present study, based only on HR-ALL patients, confirms the poor prognosis of adult patients with t(9;22) even if they are intensively treated, as found in other series.[13,41-44] In our study only the presence of t(9;22) and slow response to therapy[45] were associated with a poor prognosis in adult patients with high-risk ALL. This finding has led to the subsequent exclusion of patients with t(9;22) from the ALL-93 protocol and to the design of a specific therapeutic trial for these latter patients. In Ph^+ ALL patients the administration of combination chemotherapy including purine analogs,[46] as well as the early use of related or unrelated SCT[47-50] have provide promising results in some studies, and the value of tyrosine-kinase inhibitors has been actively investigated by several groups.[40,51,52]

The close monitoring of minimal residual disease (*BCR/ABL* rearrangement) can also contribute to better patient management.[53] The prognostic influence of additional cytogenetic changes to t(9;22) has been scarcely analyzed and some studies have demonstrated a trend to a worse prognosis for patients with these features,[40, 53] while in others the worst prognosis has been only restricted to patients with t(9;22) and loss of chromosomes, such as 9p-, -7 or 7p-.[40,54] In our series no differences in response to therapy nor in EFS and OS derived from the presence of additional cytogenetic abnormalities in adult ALL patients with t(9;22). The lack of prognostic value of t(9;22) in children with very-high risk ALL included in this study may be explained by their low number (4 cases). However, data from several studies indicate that within children with t(9;22), the adverse prognosis is restricted to those with hyperleukocytosis ($>100\times10^9$/L) and higher age (above 10 years).[40] Patients with t(4;11) and other 11q23 rearrangements were the second most frequent group of patients with structural chromosomal abnormalities in our series. As observed in large co-operative studies, patients with these cytogenetic changes are usually young and have high WBC counts.[31,55-59] Recent data indicate that ALL with 11q23 rearrangements is a heterogeneous disease group,[40] including patients with good prognosis (i.e. T-cell ALL with the t(11;19)(q23;p13.3) and *MLL/ENL* fusion), relatively favorable prognosis (i.e. B-lineage ALL with t(4;11)(q21;q23) or t(11;19)(q23;p13.3) and age 1 to 9 years), and an unfavorable prognosis (the latter groups in children aged less than one year).[60] In our series both the EFS and the OS of patients (children or adults) with 11q23 rearrangements were similar to those of the remaining groups except that of patients with t(9;22). The lack of differences in prognosis between patients with 11q23 rearrangements and the remaining non-Ph ALL subgroups could be partially explained by there being only two cases of infant ALL. In addition, these results may support data indicating that the prognosis of patients with t(4;11) or 11q23 rearrangements has been improved with the use of intensive therapy,[55-59,61] although

this should be considered with caution in our study because of the low number of 11q23 patients. In this group of patients there is evidence of the usefulness of *MLL/AF4* monitoring for the evaluation of the response to therapy.[59]

On the other hand, trisomy 8 was found in 0.4-1% of cases of ALL and seems to be associated with a poor prognosis.[13] This could not be evaluated in our series because of the low number of cases with this abnormality (two cases as isolated chromosomal change and in three associated with a t(9;22). The same occurs in patients with –7, an infrequent subgroup of patients whose prognosis is still unknown, but probably poor.[62]

We conclude that among HR-ALL patients treated with a protocol including intensive consolidation therapy as well as SCT, cytogenetic analysis at diagnosis is a useful prognostic marker, at least for adults. Adult patients with t(9;22) have the poorest outcome and new therapeutic modalities are clearly needed for this group of patients.

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JMR and JJO were primarily responsible for the design of the PETHEMA-ALL93 protocol. IG and JMHR reviewed the cytogenetic studies. JMR wrote the paper. The remaining authors qualified for authorship according to the WAME criteria, taking specific responsibility for the following parts of the content: MF for randomization of patients. AO, JMR and MF for data handling, and statistical analyses. RP, CB, CR, PB, EP, MEGV, MJM, JB, JFC, MT, JA, AM, MAS, JM, FM, EF, and JFSM performed the studies at diagnosis and followed the patients clinically.

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Disclosures

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Peer Review Outcomes

What is already known on this topic

Chromosome analysis is an important diagnostic and prognostic tool that aids risk classification and treatment randomization for patients with acute lymphoblastic leukemia (ALL). High-risk ALL is characterized by factors such as high white blood cell counts, age, poor response to initial therapy, and the presence of certain chromosomal abnormalities (in adults, the Philadelphia chromosome; in children, the Philadelphia chromosome and abnormalities of the 11q23 region).

What this study adds

This study evaluates the impact of chromosome findings in children and adults who had high-risk ALL and were treated in a multicenter prospective trial. This work confirms the association between poor outcome and the presence of a Philadelphia chromosome and blast cells in the bone marrow aspirate by day 14, although the findings involving pediatric patients were limited by the small number of cases in which recurrent chromosomal abnormalities were identified.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Susana Raimondi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Professor Raimondi and the Editors. Manuscript received July 10, 2001; accepted December 11, 2001.

Potential implications for clinical practice

With the introduction of new genetically tailored therapies into clinical practice, the identification of genetic abnormalities as markers of ALL is becoming critical for appropriate treatment selection.

Professor Susana Raimondi, Associate Editor