original paper
Haematologica 1997; 82:654-659

CENTRALIZED CYTOGENETIC ANALYSIS OF PEDIATRIC ACUTE LEUKEMIA: RESULTS OF AN ITALIAN COLLABORATIVE EXPERIENCE

LAURA SAINATI,* ANNA LESZL* MARIACATERINA PUTTI,* FRANCESCO PASQUALI,°# EMANUELA MASERATI,# EMILIO DONTI,@ GIOVANNA VENTI,@ PAOLO SIMI,^ CECILIA GIULIANI,^ ADRIANO ANGIONI,§ MARIO STELLA,** ANNA MONTALDI,** MARIO SESSAREGO,°° LUIGI ZANESCO,* ANDREA BIONDI,## GIUSEPPE BASSO@@

**Dipartimento di Pediatria, Centro Leucemie Infantili, Università di Padova; °Biologia Generale e Genetica Medica, Università di Pavia; #Centro di Genetica Clinica, Università di Sassari; @Istituto di Medicina Interna e Scienze Oncologiche, Università di Perugia; ^Laboratorio di Genetica, Istituto di Clinica Pediatrica, Università di Pisa; §Servizio Trasfusionale, Ospedale Bambino Gesù, Rome; **Servizio di Genetica Umana e Immunotrasfusionale, Ospedale S. Bortolo, Vicenza; °°Dipartimento di Medicina Interna, Università di Genova; ##Ospedale S.Gerardo, Monza; @@Dipartimento di Pediatria, Università di Torino; Italy*

ABSTRACT

Background and Objective. Cytogenetic analysis of acute leukemia yields important information which has been demonstrated to be correlated to patient survival. A reference laboratory was created in order to perform karyotype analysis on all cases of acute leukemia enrolled in the AIEOP (*Associazione Italiana Emato-Oncologia Pediatrica*) protocols.

Methods. From January 1990 to December 1995, 1115 samples of children with ALL or AML were sent in for cytogenetic analysis. The results of cell cultures were screened in the Reference Laboratory and then the fixed metaphases were sent to one of the six cytogenetic laboratories for analysis.

Results. The leukemic karyotypes of 556 patients were successfully analyzed. An abnormal clone was detected in 49% of cases of ALL and in 66% of AML. In ALL the most frequent abnormality was 9p rearrangement. Other recurrent abnormalities were t(9;22), t(4;11) and t(1;19). In AML t(8;21), t(15;17) and 11q23 rearrangement were the most frequent structural abnormalities. These findings are similar to the results obtained in other multicenter studies using a similar approach.

Interpretation and Conclusions. Our data confirm the feasibility of performing cytogenetic analysis in a centralized laboratory on mailed samples of bone marrow and/or peripheral blood; this is very important considering that cytogenetic analysis of neoplastic tissue requires a special laboratory and expert staff.

Key words: children, acute leukemias, cytogenetic, reference laboratory, chromosomes, prognosis

In recent years numerous collaborative studies have been conducted all over the world on the treatment of pediatric malignant neoplasms. The aim of these studies was to collect data on clinical and biological features in order to identify prognostic factors and different subgroups of disease with different clinical relevance to improve the treatment. Such collaborative studies have led pediatric oncology to achieve important goals in our understanding of the pathogenesis, diagnosis and treatment of neoplastic diseases. An essential premise for multicentric studies is that all cases, or at least the majority of them, can be adequately analyzed using current evaluation techniques.

The cytogenetic analysis of leukemic blasts in childhood acute lymphoblastic leukemia (ALL) has identified chromosome abnormalities in about 50-80% of cases.[1] The presence of well-defined struc-

Correspondence: Laura Sainati, MD, Dipartimento di Pediatria Via Giustiniani 3, 35128 Padua, Italy. Tel. international +39.49.8213579. Fax. international +39.49.8213510. E-mail: seguno@child.pedi.unipd.it

Acknowledgements: Principal investigators: L. Felici (Cl. Pediatrica, Ancona); N. Santoro (Cl. Pediatrica I, Bari); T. Santostasi (Cl. Pediatrica II, Bari); P. Cornelli (O. Riuniti, Bergamo); A. Pession (Cl. Pediatrica III, Bologna); A. Arrighini (Cl. Pediatrica, Brescia); G.M. Fiori (Div. Oncoematologia Pediatrica, Cagliari); R.Galanello (Cl. Pediatrica II; Cagliari); A. Sciotto (Div. Oncoematologia Pediatrica, Catania); S. Magro (Div. Ematologia, Catanzaro); A. Lippi (O. Meyer, Firenze); M. Cominetti (O. Galliera, Genova); C. Rosanda (O. Gaslini, Genova); F. Massolo, (Cl. Pediatrica II, Modena); A. Murano (Cl. Pediatrica I, Napoli); M.F. Pinta (O. Pausillipon, Napoli); S. Auricchio, (Cl. Pediatrica II, Napoli); A. Correra (O. SS. Annunziata, Napoli); G. Fugardi (Cl. Pediatrica I, Palermo); G. Izzi (Div. Ematologia Pediatrica, Parma); M. Aricò (Cl. Pediatrica, Pavia); A. Amici (Cl. Pediatrica, Perugia); R. Di Lorenzo (Div. Ematologia, Pescara); C. Favre (Cl.Pediatrica III, Pisa); F. Nobile (Div. Ematologia, Reggio Calabria); A.M. Testi (Cattedra di Ematologia, La Sapienza, Roma); B. Werner (Cl.Pediatrica, La Sapienza, Roma); D. Gallisai (Cl. Pediatrica, Sassari); D'Ambrosio (Cl. Pediatrica, Siena); E. Barisone (Cl. Pediatrica, Torino); P. Tamaro (Cl. Pediatrica, Trieste); L. Nespoli (Cl. Pediatrica, Varese); G. Marradi (Cl. Pediatrica, Verona). This work has been supported by CNR grants n° 92.02234.39; 93.02211.39; 94.01178.39; 95.00409.39 and in part by the AIRC; MURST 60%, Turin University. The authors thank the members of the Reference Laboratory Franca Bonan, Maria Grazia Giacometti, Barbara Michielotto, Chiara Parpaiola and Monica Spinelli for their skilled and essential technical work, Doctor Maria Grazia Cocito for immunophenotyping.The authors wish to thank "30 ore per la vita" – AIL.
Received May 26, 1997; accepted September 29, 1997.

tural abnormalities in children with ALL correlates with a different prognosis and with different treatment. In the latest BFM family protocol, the presence of the t(9;22) and t(4,11) translocation is considered to be an independent factor indicating a poor prognosis and prompting a high-risk therapeutic program.[1-5]

Other abnormalities, such as t(8;21), t(15;17), are associated with a particular subtype of acute myeloblastic leukemia (AML);[6] in all such cases cytogenetic results are crucial in the choice of treatment. Cytogenetic analysis is consequently standard practice nowadays in the diagnostic approach to leukemia, but very accurate culturing methods and considerable technical expertise are needed for proper karyotype analysis of acute leukemia.

Since 1990, the *Onco-Hematology Laboratory of the Pediatric Department at Padua University* has performed cytogenetic analysis on acute leukemia samples, together with diagnostic and biological studies, on request for any institution following the AIEOP protocols.

This paper presents an overview of the results of this collaborative cytogenetic study which involved several AIEOP centers and a few cytogenetic laboratories between 1990 and 1995. The aim of this paper was to establish the feasibility of centralizing cytogenetic studies and to compare the outcome of this approach with other collaborative studies.

Materials and Methods

Patients

In 1988 a tissue bank for the storage of bone marrow and peripheral blood samples of pediatric patients enrolled in the therapeutic trials of the AIEOP was created in Padua at the Onco-Hematology Laboratory, a reference laboratory where morphological evaluation and immunophenotyping were also performed.[7] A year later, all the AIEOP centers unable to perform cytogenetic investigations in their own laboratories were given the opportunity to send a heparinized bone marrow sample to the central laboratory, together with a sample for the bone marrow bank, thus avoiding any extra cost of mailing the sample for cytogenetic analysis alone.

Between January 1990 and December 1995, 1309 samples of bone marrow and/or peripheral blood from children with suspected acute leukemia were sent to Padua for cytogenetic evaluation; 1115 of these were ALL or AML at diagnosis or relapse. The other 193 samples belonged to patients with non-neoplastic diseases, and these cases were excluded from the study. The 1115 leukemia patients were enrolled in the AIEOP protocols after informed consent was obtained. There were 667 males and 448 females; their ages ranged from 1 day to 16 years.

All the cases were classified according to the French-American-British (FAB) classification[8,9] and were studied according to the minimal requirements of the BFM-family group criteria for the evaluation of childhood acute leukemia.[10]

Cytogenetic analysis

Heparinized bone marrow and/or peripheral blood samples were collected in syringes or test tubes and mailed to the laboratory at room temperature with next-day delivery, 48 hours were required for samples sent from Sicily and Sardinia. A white blood cell count of the sample was obtained, and cell vitality was checked; cultures were prepared using 1×10^6 cells/mL of medium (RPMI 1640, 20% FCS, L-glutamine and 50 ng/mL penicillin/streptomycin). When an adequate number of cells was available, three different cultures (for direct harvest, overnight exposure to colchemid and 24-hour culture) were prepared. In the cases of AML, an additional culture was incubated for 3 days with a supernatant of a cell line with growth factors.[11] Any samples without vital cells, or those with fewer than 5×10^6, or with EDTA used as an anticoagulant, were classified as *unsuitable* and excluded from the analysis. Routine methods were used for culture harvesting and chromosome preparation. The results of cell culture were screened in the reference laboratory.

The cell suspensions containing analyzable metaphases were sent to one of the cytogenetic laboratories in Genoa (MS), Pavia (FP, EM), Perugia (ED), Pisa (PS, CG), Rome (AA), or Vicenza (MS, AM), where cytogenetic analysis was performed using routine methods. From 1 to 3 slides were screened in each case and 10-20 metaphases were analyzed. Chromosomes were identified and assigned according to the *International System for Human Cytogenetic Nomenclature*.[12] Cell culture failure was defined as cases where fewer than 10 well-analyzable metaphases were found. The cytogenetic results were resubmitted to the reference laboratory, where all data were recorded and sent back to the center to which the patient belonged.

Results

The 1115 samples of acute leukemia analyzed included: 951 cases at diagnosis (802 ALL and 149 AML) and 164 at relapse (134 ALL and 30 AML). All the patients joined the AIEOP protocols for childhood ALL or AML. The number of AIEOP centers requesting cytogenetic analysis has progressively increased over the years, from 12 in 1990 to 27 in 1995.

Of the 1115 samples received, 391 (35%) were unsuitable for cytogenetic analysis due to the low number of cells for cultures (< 5 million), the lack of vital cells in the sample, clotting or the use of an unsuitable anticoagulant; these samples were consequently excluded from the study (*unsuitable* sam-

ples). A karyotype was not definite in 15% of the cases because a very low mitotic index or poor-quality metaphases were obtained from the cell culture (168 cell culture failures).

The proportion of suitable samples did not vary consistently over the years; this proportion derives from the inadequacy of the samples delivered in some instances.

In ALL, unsuitable samples were the main reason for the failure of the cytogenetic analysis (40%) (Table 1a), whereas in the AML they amounted to 15% (Table 1b).

We successfully analyzed the leukemic karyotype of 556 patients, identifying 296 cases with an abnormal clone. In ALL these results were almost all obtained from direct harvesting and overnight cultures, the latter being the most successful. In AML, the results were obtained from overnight cultures and from cultures with added growth factors, as described in the methods.

In the ALL group, the percentage of cases with an abnormal clone varied over the years between 35% and 60% (Table 2). In the AML group, an abnormal clone was observed in 53% and 77% of the cases (Table 3). In the last three years, an abnormal clone was always detected in more than 50% of ALL. Table 4 shows the distribution based on the immunophenotype of the recurrent structural abnormalities found in ALL at diagnosis. The most frequent abnormality in our series was the rearrangement of the short arm of chromosome 9, which was found in 14 cases with either the B or the T phenotype. Twelve cases presented t(9;22), 8 of these were C-ALL, 1 prepreB, 2 preB and 1 had a hybrid phenotype; t(1;19) was detected in 8 cases, all of which were preB ALL; t(4;11) was detected in 6 cases, 4 of which were C-ALL, 1 was a prepre B ALL and 1 presented a hybrid phenotype.

Table 5 shows the ploidy distribution by immunophenotype in ALL at diagnosis in our series.

The most frequent group in the cases with abnormal karyotypes is the pseudodiploid (70 cases, 21%); a hyperdiploid karyotype with more than 50 chromosomes was found in 37 cases (11%), and with 47 to 50 chromosomes in 30 cases (9%). A hypodiploid karyotype was observed in 19 cases (5%).

Tables 6 and 7 show the distributions of recurrent structural abnormalities and ploidy according to FAB subgroups in AML at diagnosis. The most frequent structural rearrangements were the reciprocal translocations t(8;21) in the M1/M2 (11 cases) and t(15;17) in the M3 (12 cases) FAB sub-

Table 1. Results of centralized cytogenetic analysis.

a. ALL results

	1990	*1991*	*1992*	*1993*	*1994*	*1995*	*Total cases*
Unsuitable samples	21 (33%)	46 (49%)	45 (32%)	72 (36%)	90 (42%)	91 (40%)	365 (40%)
Cell culture failure	13 (21%)	8 (9%)	29 (21%)	26 (13%)	40 (19%)	33 (15%)	149 (16%)
Suitable cases	29 (47%)	39 (42%)	65 (47%)	104 (51%)	83 (39%)	103 (45%)	423 (44%)
Total cases	63 (100%)	93 (100%)	139 (100%)	202 (100%)	213 (100%)	227 (100%)	937 (100%)

b. AML results

	1990	*1991*	*1992*	*1993*	*1994*	*1995*	*Total cases*
Unsuitable samples	3 (14%)	3 (16%)	2 (9%)	7 (18%)	7 (20%)	4 (9%)	26 (15%)
Cell culture failure	2 (9%)	3 (16%)	1 (5%)	5 (13%)	6 (17%)	2 (5%)	19 (11%)
Suitable cases	17 (77%)	13 (68%)	18 (86%)	26 (69%)	22 (63%)	37 (86%)	133 (74%)
Total cases	22 (100%)	19 (100%)	21 (100%)	38 (100%)	35 (100%)	43 (100%)	178 (100%)

Cases were classified as "unsuitable" or as "cell culture failures" according to the criteria mentioned in the "Materials and Methods" section.

Table 2. Proportion of ALL cases with normal and abnormal karyotypes per year.

	1990	*1991*	*1992*	*1993*	*1994*	*1995*	*Total cases*
Normal	18 (62%)	23 (59%)	42 (65%)	42 (40%)	42 (50%)	48 (47%)	215 (51%)
Abnormal	11 (38%)	16 (41%)	23 (35%)	62 (60%)	41 (50%)	55 (53%)	208 (49%)
Total	29 (100%)	39 (100%)	65 (100%)	104 (100%)	83 (100%)	103 (100%)	423 (100%)

Table 3. Proportion of AML cases with normal and abnormal karyotypes per year.

	1990	*1991*	*1992*	*1993*	*1994*	*1995*	*Total cases*
Normal	8 (47%)	3 (23%)	6 (33%)	10 (38%)	6 (27%)	12 (32%)	45 (34%)
Abnormal	9 (53%)	10 (77%)	12 (67%)	16 (62%)	16 (73%)	25 (68%)	88 (66%)
Total	17 (100%)	13 (100%)	18 (100%)	26 (100%)	22 (100%)	37 (100%)	133 (100%)

Table 4. Recurrent cytogenetic abnormalities by immunophenotype in ALL at diagnosis 1990-95.

	No. of cases	pre-preB	preB	B	C-ALL	T	Hybrid	nd
t(1;19)(q23;p13)	8	–	8	–	–	–	–	–
t(9;22)(q34;q11)	12	1	2*	–	8	–	1	–
t(4;11)(q21;q23)	6	1	–	–	4§	–	1	–
der(11)(q23)	2	–	–	–	2	–	–	–
t(8;14)(q24;q32)	1	–	–	1	–	–	–	–
der(14)(q11-q13)∑	5	–	–	–	–	5	–	–
t(7;7)(p15;q36)	1	–	–	–	–	1	–	–
der(6)(q21-q23)	6	–	2	–	3	–	–	1
del(9)(p21)/der(9p)	14	2	5	–	2	4	–	1
der(12)(p)	4	–	–	–	2	1	1	–
-20/20q-	9	–	3	–	6	–	–	–
+21^	4	–	–	–	4	–	–	–

*•t(1;14),t(11;14),t(8;14); * variant translocations t(9;20;22)(q34;?;q11), t(8;22)(q24;q11); § variant translocation t(2;4;11)(q14;q21;q23); ^unassociated with other numerical abnormalities in non-Down patients; nd: immunophenotype not defined.*

Table 5. Distribution of immunophenotype by ploidy in ALL at diagnosis 1990-95.

	No. of cases	pre-preB	preB	B	C-ALL	T	Hybrid	nd
Hyperdiploid (>50)	37 (11%)	–	7	24	–	–	–	6
Hyperdiploid (47-50)	30 (9%)	1	9	14	–	5	–	1
Diploid (normal)	188 (54%)	1	54	79	1	24	6	23
Pseudodiploid	70 (21%)	2	16	30	2	12	3	5
Hypodiploid	19 (5%)	–	3	12	–	2	–	2
Total	344	4	89	159	3	43	9	37

nd: immunophenotype not defined.

groups. A rearrangement with a breakpoint in 11q23 was detected in 7 cases; 1 case showed a rearrangement of chromosome 11 at q21. Isolated trisomy 8 and 21 were documented in 10 and 6 cases, respectively. Monosomy 7 was found in 4 cases. On the whole, our results demonstrate that the most frequent clonal karyotype alteration in AML was pseudodiploidy, detected in 45% of cases, while metaphases with a number of chromosomes between 47 and 50 were found in 15% of cases; few cases presented a hypodiploid (3%) or hyperdiploid (2%) karyotype with more than 50 chromosomes.

Discussion

Since several multicenter studies on childhood acute leukemia have been conducted all over the world, we now have a considerable number of equally-studied and homogeneously-treated cases. Most of our understanding of the biology and treatment of leukemias comes from such cooperative studies in children.

Since 1990, the reference laboratory for all the AIEOP centers has performed cytogenetic analysis and all other diagnostic and biological procedures on request.

Collecting many samples at a single laboratory reduces the costs and enables hospitals with no cytogenetic laboratory of their own to have this analysis done inexpensively. The addition of the cytogenetic analysis facility to a pre-existing biological bank has meant a further reduction in costs and an easier procedure for the doctor responsible for the patients and who requests the analysis. Over

Table 6. Recurrent cytogenetic abnormalities by FAB groups in AML at diagnosis 1990-95 .

	No. of cases	M0	M1	M2	M3	M4	M5	M6	M7
t(8;21)(q22;q22)	11	–	3	8	–	–	–	–	–
t(15;17)(q22;q12)	12	–	–	–	12	–	–	–	–
der(11)(q23)	2	–	–	–	–	2	–	–	–
t(9;11)(p22;q23)	2	–	–	–	–	1	1	–	–
t(11;19)(q23;p13)	2	–	–	–	–	–	2	–	–
t(8;16)(p11;p13)	2	–	–	–	–	–	2	–	–
t(10;11)(p13;q21)	1	–	–	–	–	–	1	–	–
inv(16)(p13q22)	1	–	–	–	–	1	–	–	–
der(6)(q)	2	1	1	–	–		–	–	–
t(6;9)(p23;q34)	2	–	1	–	–	1	–	–	–
t(9;22)(q34;q11)	1	–	1	–	–	–	–	–	–
t(4;11)(q21;q23)	1	–	1	–	–	–	–	–	–
del(9)(q22)	2	–	–	2	–	–	–	–	–
+8*	10	1	1	1	1	2	3	1	–
+21*	6	–	3	1	–			1	1
–7	4	2	1	–	–	1	–	–	–
Others	14	3	4	1	–	4	–	1	1

**unassociated with other numerical abnormalities in non-Down patients.*

Table 7. Distribution of FAB groups by ploidy in AML at diagnosis 1990-95.

	No. of cases (%)	FAB groups M0	M1	M2	M3	M4	M5	M6	M7	nd
Hyperdiploid (>50)	3 (2%)	–	1	–	–	–	2	–	–	–
Hyperdiploid (47-50)	17 (15%)	–	5	2	1	2	3	1	1	2
Diploid (normal)	38 (35%)	1	7	2	4	4	10	–	3	7
Pseudodiploid	49 (45%)	3	8	7	11	8	9	1	–	2
Hypodiploid	4 (3%)	–	1	1	–	1	–	–	–	1
Total	111	4	22	12	16	15	24	2	4	12

nd: FAB not defined.

the years, the number of samples sent to our laboratory has increased progressively, demonstrating the value of this organization in Italy at this time. The slides with chromosome preparations were split between different cytogenetic laboratories in order to reduce the response time.

A considerable number of samples were lost due to incorrect sampling and preserving methods. The most frequent causes were: faulty cell harvesting methods at bone marrow aspiration; the use of EDTA as an anticoagulant; coagulation of the sample; and contamination of the blood. The percentage of unsuitable samples is higher in the ALL group, probably due to the difficulty in retrieving a large number of blasts by bone marrow aspiration at diagnosis in a consistent number of these leukemia cases, and to problems specific to ALL samples. The percentage of unsuitable samples has not improved with time, possibly because the number of centers sending the samples is increasing, so new centers taking part in the study every year may be responsible for our failure to contain this waste of material. On the other hand, given the large number of suitable samples submitted for cytogenetic analysis, our experience confirms the feasibility of performing cytogenetic analysis on mailed samples of leukemic cases, as reported in the BFM experience.[5] The percentage of successful cultures with correct sampling and preserving methods ranged from 68% to 83% for the ALL cases (a total of 672 cases) and from 79% to 95% for the AML cases (a total of 152 cases).

An abnormal karyotype was detected in 208 cases of ALL (49%) and in 88 cases of AML (66%). These results are similar to the findings of a BFM multicenter study conducted on 1843 children with acute leukemia.[2] Lampert reports a cytogenetic success in 55-60% of ALL and in 70% of AML, with a proportion of abnormal karyotypes of 60% in ALL and 68% in AML. The percentage of samples with no metaphases, or with a normal karyotype, is reportedly lower among most of the single-center groups with extensive experience than it is in our series,[13] though it is not very dissimilar from other reports.[14] In the French cooperative study on karyotypes in childhood leukemia, clonal abnormality was found in a higher percentage of ALL (71%); but this collaboration was organized differently from ours, i.e. in the French cooperative study, karyotype analysis was performed by each hospital and then the karyotypes were reviewed by all those taking part in the group.[15] On the other hand, the existence of a central laboratory gives everyone the opportunity to have cytogenetic investigations performed, and thus retrieves many samples that would otherwise be lost for the purposes of cytogenetic analysis. It is also worth noting the fact that our efficiency in detecting abnormal clones has improved in the latter years of our work (Tables 2 and 3), both in ALL and in AML, and a more careful handling of the samples should enable us to improve on the standard of results.

The distribution of the clonal abnormalities detected in ALL at diagnosis (Table 5) is similar to the one of the German cooperative study.[2]

At this stage, we cannot discuss the leukemic karyotype in detail, since the aim of this study was to verify the feasibility of a cytogenetic cooperative study for analyzing childhood acute leukemia.

Our results indicate that overnight exposure to colchemid gives the best chances of success in ALL, in terms of metaphases; whereas in AML, evaluable metaphases are obtained from the three short-term cultures, as the addition of growth factors, is useful. In conclusion, we believe that our data confirm the feasibility of using a centralized laboratory to carry out cytogenetic analysis on mailed samples of bone marrow and/or peripheral blood of acute leukemia. This approach is not only an easy, inexpensive solution for gaining complete information in multicenter studies, but it also creates easily-accessible storage of material and information available for further studies, being the base of future investigations. Some cytogenetic abnormalities with recognized clinical significance can now be screened using a molecular biology method,[16,17] but others with variant or as of yet unknown structures cannot be identified by molecular biological methods. It is also very important to continue looking for other abnormalities that might be relevant in the diagnosis, treatment and follow-up of leukemia,[18,19] confirming the relevance of cytogenetic analysis in acute leukemia.

Our data confirm the feasibility of cytogenetic analysis performed in a centralized laboratory on mailed samples of bone marrow and/or peripheral blood of acute leukemias; this could be a very helpful approach in multicenter studies, overcoming the bias of the limited number of laboratories available for cytogenetic analysis. In fact, the cytogenetic analysis of the neoplastic tissue requires a special laboratory with an expert staff.

References

1. Pui CH, Crist WM, Look AT. Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. Blood 1990; 76:1449-62.
2. Lampert F, Harbott J, Ritterbach J. Chromosomen aberrationen bei akuten Leukamien in Kindesalter: Analyse von 1009 Patienten. Klin Pädiatr 1991; 203:311-8.
3. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996; 14:18-24.
4. Pui CH, Crist WM. Cytogenetic abnormalities in childhood acute lymphoblastic leukemia correlates with clinical features and treatment outcome. Leuk Lymphoma 1992; 7:259-74.
5. Katz JA, Taylor LD, Caroll A, Elder FFB, Mahoney DH. Cytogenetic features of childhood acute lymphoblastic leukemia. A concordance study and a pediatric oncology group study. Cancer Genet Cytogen 1991; 55:249-56.
6. Second MIC Cooperative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukaemias. Cancer Genet Cytogen 1988; 30:1-15.
7. Basso G, Biondi A, Cantù-Rajnoldi A. Biologia delle leucemie.

Rapporto tra l'aspetto biologico e l'applicazione clinica. Riv Ital Pediatr 1993;19:(Suppl 6)11-7.
8. Bennet JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukemias. Br J Haematol 1976; 33:451-8.
9. Bennet JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia. Ann Intern Med 1985; 103:626-9.
10. Van der Does van den Berg A, Bartram CR, Basso G, et al. Minimal requirements for the diagnosis, classification, and evaluation of childhood acute lymphoblastic leukemia (ALL) in the "BFM family" cooperative group. Med Pediatr Oncol 1992; 20:497-505.
11. Vellenga E, Ostapovicz D, O'Rourke B. Effects of recombinant IL-3, GM-CSF and G-CSF and the proliferation of leukemic clonogenic cells in short-term and long-term culture. Leukemia 1987; 1:584-9.
12. Mitelman F. ISCN (1995): An International System for Human Cytogenetic Nomenclature. Basel: S. Karger, 1995.
13. Pui CH, Dodge RK, Look AT, et al. Risk of adverse events in children completing treatment for acute lymphoblastic leukemia: St. Jude total therapy studies. J Clin Oncol 1991; 9:1341-7.
14. Petkovic I, Josip K, Nakic M, Kastelan M. Cytogenetic, cytomorphologic and immunologic analysis in 55 children with acute lymphoblastic leukemia. Cancer Genet Cytogen 1996; 88:57-65.
15. Group Francais de Cytogenetique Hematologique. Collaborative study of karyotypes in childhood acute lymphoblastic leukemias. Leukemia 1993; 7:10-9.
16. Santini V, Zoccolante A, Bosi A, et al. Detection of bcr/abl transcripts by RT-PCR and their colorimetric evaluation in chronic myeloid leukemia patients receiving allogeneic bone marrow transplantation. Haematologica 1996; 81:201-7.
17. Cuneo A, Ferrant A, Michaux-JL, et al. Philadelphia chromosome-positive acute myeloid leukemia: cytoimmunologic and cytogenetic features. Haematologica 1996; 81:423-7.
18. Anonymous. Towards an increasingly molecular-based, patient-oriented treatment of acute myeloid leukemia [editorial]. Haematologica 1996; 81:1-2.
19. Lerede T, Bassan R, Rossi A, et al. Therapeutic impact of adult-type acute lymphoblastic leukemia regimens in B-cell/L3 acute leukemia and advanced-stage Burkitt's lymphoma. Haematologica 1996; 81:442-9.