

Karyotyping, FISH, and PCR in Acute Lymphoblastic Leukemia

Competing or Complementary Diagnostics?

Louise Olde Nordkamp, BSc,* Clemens Mellink, PhD,† Ellen van der Schoot, MD, PhD,‡ and Henk van den Berg, MD, MMed PhD*

Background: Chromosomal abnormalities, such as t(9;22)(q34;q11) (*ABL/BCR*), t(12;21)(p13;q22) (*TEL/AML1*), and t(11q23) (*MLL*) are independent prognostic indicators in childhood acute lymphoblastic leukemia resulting in risk adapted therapy. Accurate and rapid detection of these abnormalities is mandatory, which is achieved by karyotyping, fluorescence in situ hybridization, and real time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR). For cost-effective diagnostic approaches knowledge of diagnostic accuracy of these tests is required. Therefore, we aimed to determine the diagnostic accuracy of karyotyping, fluorescence in situ hybridization, and RQ-PCR analysis.

Procedure: Retrospective study conducted between January 1, 1992 and January 1, 2007 in the Emma Children Hospital in Amsterdam. All consecutive patients under 18 years with acute lymphoblastic leukaemia were included. Diagnostic tests were performed according to international standards.

Results: Diagnostic techniques show a high-reciprocal agreement and have a high-individual diagnostic accuracy in detecting the above-mentioned chromosomal translocations. However, the sensitivity of karyotyping for detecting the *TEL-AML1* fusion gene and the sensitivity of RQ-PCR for detecting *MLL*-rearrangements was rather low.

Conclusions: Diagnostic accuracy of tests for detecting t(9;22), t(12;21), and t(11q23) is generally high, although sensitivity is not optimal for all anomalies. Despite the high-diagnostic accuracy, all diagnostic techniques should be used complementary, because any detection of a (significant) chromosomal aberration irrespective of diagnostic mode has to be considered in therapy.

Key Words: acute lymphoblastic leukemia, karyotyping, FISH, RQ-PCR, cytogenetics

(*J Pediatr Hematol Oncol* 2009;00:000–000)

Leukemia's constitute approximately one-third of all malignancies in children (age 0 to 14y) and 10% in adolescents (age 15 to 19y). Of these, acute lymphoblastic leukaemia (ALL) is the most prominent type.¹ Like in

many malignancies; genetic rearrangements (such as numerical and/or structural chromosome anomalies or gene mutations) are rule rather than exception. The 3 major structural chromosomal aberrations in childhood ALL are the cytogenetically cryptic translocation t(12;21)(p13;q22), leading to a fusion of the *TEL* – (or *ETV6* –) gene and the *AML1* – (or *RUNX1* –) gene, the translocation t(9;22)(q34;q11) (resulting in a *BCR-ABL* fusion gene), and rearrangements involving the *MLL* gene on 11q23 (mostly translocations) of which the t(4;11) resulting in *MLL-AF4* fusion is the most frequent. The first translocation mentioned is an independent prognostic indicator for good prognosis, whereas the last 2 anomalies are linked with a poor prognosis in childhood ALL.² In many protocols the treatment of patients is stratified on the basis of the clinical features and chromosomal findings.³

When available, karyotyping, fluorescence in situ hybridization (FISH) and real time quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) are nowadays routinely used to detect genetic abnormalities. But all 3 techniques are either time-consuming and/or expensive. As these 3 tests provide information on similar anomalies it would be of interest to delineate the value of each test separately. Therefore, the aim of this study is to determine the diagnostic accuracy of conventional karyotyping, FISH, and RQ-PCR.

MATERIALS AND METHODS

Study Population

This retrospective study was conducted in the Department of Paediatric Oncology at the Emma Children Hospital AMC (Amsterdam). All consecutive patients under the age of 18 years at the moment of diagnosis and treated for ALL between January 1, 1992 and January 1, 2007 were included in this study. Patients with a bone marrow blast count less than 25% and/or with a type of leukemia other than progenitor B-cell or T-cell ALL were excluded. For patients who had a relapse of ALL, only the findings at initial diagnosis were considered.

Diagnosis

The diagnosis ALL was based on the presence of > 25% blasts in the bone marrow on marrow smears. In most cases conventional karyotyping was carried out including FISH and RQ-PCR for the mentioned translocations. Karyotyping and FISH analysis were performed in the Cytogenetics Laboratory of the Department of Clinical Genetics (Academic Medical Center, Amsterdam). For

Received for publication April 1, 2008; accepted August 16, 2009.

From the *Department of Paediatric Oncology, Emma Children Hospital AMC; †Department of Clinical Genetics, Academic Medical Center; and ‡Department of Experimental Hematology, Sanquin, Amsterdam, The Netherlands.

Reprints: Henk van den Berg, MD, MMed, PhD, Department of Paediatric Oncology, Emma Children Hospital Academic Medical Center, University of Amsterdam, PO Box 22700, Amsterdam 1100 DD, The Netherlands (e-mail: h.vandenberg@amc.uva.nl).

Copyright © 2009 by Lippincott Williams & Wilkins

karyotyping routine cytogenetic procedures were applied (as described in Rooney and Czepulkowski).⁴ Chromosomes were Q-banded for identification. When available, a number of 20 to 30 metaphases were analyzed. FISH was carried out by standard protocols⁵ and hybridization procedure was modified according to the probe manufacturer (Vysis-Abbott Molecular Inc, Des Plaines, IL). Probes used were LSI BCR/ABL ES (dual color translocation probe with extra signal), LSI TEL/AML1 ES (dual color translocation probe with extra signal), and LSI MLL (dual color break apart rearrangement probe). At least 200 interphase nuclei were analyzed and the number of abnormal nuclei was expressed as a percentage of the total number scored. The cut-off value for a positive result was 5%. In a small subset of patients, mainly those diagnosed before 1999, other FISH probes were used such as paint 12 for the detection of t(12;21). In this case at least 10 metaphases were examined for abnormalities.

RQ-PCR was performed at the Department of Experimental Hematology at Sanquin Diagnostics (Amsterdam). RQ-PCR was carried out according to international standards.⁶ The t(9;22), t(12;21), and t(4;11) were tested with the corresponding fusion gene transcripts (BCR-ABL m-bcr and M-bcr fusion gene transcripts, TEL-AML1 fusion gene transcript, and MLL-AF4 fusion gene transcript). The reaction was performed in a RQ-PCR.⁶ In each assay positive controls were included consisting of 10³ copies and 10 copies of a plasmid DNA calibrator containing the target gene sequences.⁶ The latter control served as a sensitivity control. The negative controls concerned no-amplification controls, which contained cDNA derived from mononuclear cells from healthy individuals, and no-template controls, which contained water instead of human cDNA. All negative controls were performed in duplicate.

Study Design

The retrospective clinical and laboratory data were collected from both digital and paper files. Data on karyotyping and FISH determinations were extracted from the central digital database at the Department of Clinical Genetics; data on RQ-PCR was extracted from the databank at Sanquin.

On the basis of the type of research namely retrospective analysis of stored data, permission of Medical Ethical Committee was not needed.

Statistical Analysis

All data were analyzed using SPSS 12.0.1 (SPSS Inc, Chicago, IL). Nominal data were analyzed by using a χ^2 test; for ordinal data the Mann-Whitney test was applied. The *P* values < 0.05 were considered statistically significant.

For comparison of diagnostic techniques (karyotyping, FISH, or RQ-PCR) only those cases were analyzed for which at least results of 2 techniques were available. To exclude biases, cases in which at least 2 tests were carried out were compared with cases in which the results of less than 2 tests were available.

To compare diagnostic tests to each other, the resemblance in results between 2 techniques per aberration was measured by Cohen kappa coefficient (κ). Although standards are not permitted strictly speaking, κ agreement was considered: poor $\kappa < 0.20$; fair $0.21 \leq \kappa \leq 0.40$; moder-

ate $0.41 \leq \kappa \leq 0.60$; substantial $0.61 \leq \kappa \leq 0.80$; and good $\kappa > 0.80$.⁷

Validity of a test was quantified by calculating sensitivity. For this assessment, it was necessary to compare the results with a gold/reference standard. As for detecting chromosomal anomalies such a reference standard is lacking, we composed a virtual golden standard. This virtual standard was composed of all patients with a positive result in any of the diagnostic tests mentioned before. In this way, the sensitivity is more relevant and in line with clinical practice, where in most cases a single positive test fulfils the criterion to be included in the specific patient category. Using this virtual golden standard specificity, positive predictive value, and likelihood ratio of a positive test became irrelevant, that is 100%,100% and infinite.

RESULTS

Population

From January 1, 1992 to January 1, 2007 306 patients were treated for ALL in our institution. Three patients were excluded because they had less than 25% blasts in their bone marrow and 1 patient was excluded because he had a B-cell leukemia. The characteristics of the remaining 302 patients with ALL are displayed in Table 1. Results of cytogenetic investigations with respect to t(9;22), t(12;21), and 11q23 are given in Table 2.

Cytogenetic Investigations: Conventional Karyotyping

Karyotyping was performed on 245 (81%) patients. In this group, in 54 patients (22%) a normal karyotype was

TABLE 1. General Characteristics of Patients Used in This Study

	All Patients
Male (%)	177 (59%)
Median age (IQR)/(min-max)	4 (2-8)/(0-17)
Immunophenotype	
Progenitor B cell (%)	198 (66%)
T cell (%)	38 (13%)
Unknown (%)	66 (22%)
Extra medullar leukemia (%)	43 (14%)
CNS (%)	19 (6.3%)
White blood cell count	
< 10·10 ⁹ (%)	132 (44%)
10-50·10 ⁹ (%)	90 (30%)
50-100·10 ⁹ (%)	30 (9.9%)
≥ 100·10 ⁹ (%)	48 (16%)
Unknown (%)	2 (0.7%)
Event-free survival	
Yes (%)	219 (73%)
No (%)	82 (27%)
Unknown (%)	1 (0.3%)
Median event-free survival	70 mo
Secondary malignancy	
Yes (%)	7 (2.3%)
No (%)	295 (98%)
Died	
Yes (%)	65 (22%)
No (%)	236 (78%)
Unknown (%)	1 (0.3%)
Total (%)	302 (100%)

CNS indicates central nervous system; IQR, interquartile range, max, maximum; min, minimum.

TABLE 2. Test Characteristics of Karyotyping, FISH, and RQ-PCR in the Detection of *BCR-ABL*, *TEL-AML1*, and *MLL*-rearrangements

Karyotyping	<i>BCR-ABL</i>	<i>TEL-AML1</i>	<i>MLL</i>
Total	302	302	302
Not tested/no metaphases	57	57	57
Tested (%)	245 (81%)	245 (81%)	245 (81%)
Normal (%)	241 (98%)	243 (99%)	234 (96%)
Positive (%)	4 (1.6%)	2 (0.8%)	11 (4.5%)
FISH			
Total	302	302	302
Not tested/failed (%)	181 (60%)	171 (57%)	199 (66%)
Tested (%)	121 (40%)	131 (43%)	103 (34%)
Normal (%)	117 (97%)	97 (74%)	96 (93%)
Positive (%)	4 (3.3%)	34 (26%)	7 (6.8%)
RQ-PCR			
Total	302	302	302
Not tested/failed (%)	150 (50%)	169 (56%)	160 (53%)
Tested (%)	152 (50%)	133 (44%)	142 (47%)
Normal (%)	148 (97%)	100 (75%)	139 (98%)
Positive (%)	4 (2.6%)	33 (25%)	3 (2.1%)

FISH indicates fluorescence in situ hybridization; RQ-PCR, real time quantitative reverse transcriptase polymerase chain reaction.

found. Numerical abnormalities were observed in 122 (50%) patients, ALL specific structural chromosomal aberrations, including t(9;22), t(12;21) and rearrangements involving 11q23 were observed in 85 (35%) patients. Furthermore, deletions of 6q were observed in 24 (9.8%) patients. Involvement of the *MLL* gene in a translocation occurred in 11 patients (4.5%), whereas the t(9;22) and t(12;21) were less frequently observed [4 (1.6%) and 2 patients (0.8%), respectively].

Cytogenetic Investigations: FISH

Owing to the later introduction of FISH as a structural tool for ALL diagnostics only 43% of cases were tested by FISH. On the basis of the availability of probes over time, only in 34% the full probe set (*BCR-ABL*, *MLL*, and *TEL-AML1*) was applied. *TEL-AML1* was tested in 131 cases (43%); 34 were found positive (26%). The *BCR-ABL* aberration and rearrangements in the *MLL* gene were discovered in only a small percentage of the patients that had been tested; that is, 4/121 or 3.3% and 7/103 or 6.8%, respectively.

RQ-PCR

RQ-PCR was performed more often than FISH (44% to 50% of the patients had been tested). Rearrangements of the *BCR-ABL* fusions were observed in 2.6% of the tested patients, whereas *MLL-AF4* fusions were observed in 2.1%.

Comparison of the Diagnostic Tests

For the detection of the aberrations, not all 3 diagnostic techniques were used in every patient. This was due to availability of the test in relation to the date of diagnosis and also to eventual failures of the tests (such as absent metaphases in the slides or limited number of cells available). No significant differences ($P > 0.05$) were observed in sex, age, white blood count, and secondary malignancy between the patients tested with at least 2 test modalities and the patients that have been tested using only one test modality (often only karyotyping). Patients from the latter group were mostly diagnosed before 1999. Before 1999,

98% was not tested with FISH and 78% was not tested with RQ-PCR for t(9;22) (vs. 14% and 15% after 1999); 91% was not tested with FISH and 87% not with RQ-PCR for t(12;21) (vs. 15% and 18% after 1999); and 100% was not tested with FISH and 83% was not with RQ-PCR for *MLL*-rearrangements (vs. 24% and 16% after 1999). This group of patients diagnosed before 1999 significantly differed from the first group in the number of patients with extra medullar leukemia or central nervous system involvement, the period of event-free survival, the percentage of deceased patients and the phenotype (mean $P = 0.008$).

BCR-ABL Fusion

Comparison of karyotyping, FISH results, and RQ-PCR data were in line with each other demonstrated by κ values of 0.85 (karyotyping vs. FISH), 1.00 (karyotyping vs. RQ-PCR), and 1.00 (FISH vs. RQ-PCR), respectively. The diagnostic accuracy of the diagnostic tests for the detection of t(9;22) was classified good as well, expressed by high sensitivities of 80% to 100% and high negative predictive values for the individual tests. The results of the individual tests and the results of the diagnostic accuracy are displayed in Tables 3 and 4, respectively.

TEL-AML1 Fusion

Karyotyping had a poor agreement with FISH and RQ-PCR ($\kappa = 0.11$ and $\kappa = 0.06$, respectively) with respect to the detection of the *TEL-AML1* fusion. FISH and RQ-PCR displayed a good agreement ($\kappa = 0.93$). Karyotyping failed to detect a considerable part of the *TEL-AML1* translocations (sensitivity 6%), as expected since the translocation is cytogenetically cryptic, whereas both FISH and RQ-PCR were highly sensitive (94% and 97%, respectively).

MLL Rearrangements

In detecting *MLL* rearrangements karyotyping and FISH agreed substantially ($\kappa = 0.79$), whereas RQ-PCR corresponded fairly with FISH and moderately to karyotyping ($\kappa = 0.24$ and $\kappa = 0.59$, respectively). Karyotyping and FISH had high sensitivities for detecting *MLL* rearrangements (85% and 100%, respectively), whereas RQ-PCR showed a low sensitivity of 30%. The RQ-PCR was applied for the detection of the *MLL-AF4* fusions only. When the karyotypic and the RQ-PCR results were compared for the translocations only, the agreement was good ($\kappa = 0.85$).

DISCUSSION

Detection of chromosomal anomalies in the malignant cells in ALL is of major importance for defining risk for relapse and for stratification in specific treatment groups.³ In this study, the sensitivity of karyotyping, FISH, and RQ-PCR-analysis for the detection of 3 specific chromosomal translocations t(12;21)(p13;q22), t(9;22)(q34;q11), and t(11q23) generally proved to be high. However, the sensitivity of karyotyping in detecting the *TEL-AML1* translocation was found to be low (6%), as expected as this is a cytogenetically cryptic translocation. The ones observed are probably those, which have additional *TEL* deletions on the other chromosome 12. This is in line with reports in literature.⁸⁻¹² The low sensitivity of RQ-PCR in detecting rearrangements in the *MLL* gene (30%) can be explained as we only tested for the *MLL-AF4* fusions.

TABLE 3. Overview of the Results of Karyotyping, FISH, and PCR in the Tested Aberrations

<i>BCR-ABL</i>	No. Cases	Karyotyping	FISH	PCR
Karyotyping +	4		3/4 detected 0/4 not detected 1/4 FISH not performed	4/4 detected 0/4 not detected 0/4 PCR not performed
FISH +	4	3/4 detected 1/4 not detected 0/4 karyotyping failed		3/4 detected 0/4 not detected 1/4 PCR not performed
PCR +	4	4/4 detected 0/4 not detected 0/4 karyotyping failed	3/4 detected 0/4 not detected 1/4 FISH not performed	
<i>TEL-AML1</i>				
Karyotyping +	2		2/2 detected 0/2 not detected 0/2 FISH not performed	1/2 detected 0/2 not detected 1/2 PCR not performed
FISH +	34	2/34 detected 26/34 not detected 6/34 karyotyping failed		28/34 detected 1/34 not detected 5/34 PCR not performed
PCR +	33	1/33 detected 26/33 not detected 6/33 karyotyping failed	28/33 detected 2/33 not detected 3/33 FISH not performed	
<i>MLL aberrations</i>				
Karyotyping +	11		4/11 detected 0/11 not detected 7/11 FISH not performed	3/11 detected 4/11 not detected 4/11 PCR not performed
FISH +	7	4/7 detected 2/7 not detected 1/7 karyotyping failed		1/7 detected 6/7 not detected 0/7 PCR not performed
PCR +	3	3/3 detected 0/3 not detected 0/3 karyotyping failed	1/3 detected 0/3 not detected 2/3 FISH not performed	

FISH indicates fluorescence in situ hybridization; PCR, polymerase chain reaction.

Karyotyping is a powerful technique that provides a global picture of the entire genetic constitution of a cell (with restrictions due to resolution and provided that the aberrant cell population has divided under the used culture conditions). In general, routine cytogenetic analysis detects all numerical and (large) structural chromosomal anomalies. If only target-specific techniques such as FISH and RQ-PCR are used, quite a number of chromosomal unbalances would have been missed.¹³ Karyotyping in ALL is notoriously difficult due to the low-mitotic index and often poor quality of the obtained metaphases. Also, interpretation of the findings may be difficult if the

karyotype is complex and/or ill-defined or when multiple clones are present.^{14,15} Taking all this in consideration, therapeutic and prognostic consequences, however, still rely for a great part on karyotypic findings.¹⁶

In this study, karyotyping discovered 80% of the *BCR-ABL* translocations. In the missed case, the *BCR-ABL* fusion either is a submicroscopic rearrangement or is camouflaged as a result of a complex karyotype. The translocation t(12;21) is cryptic and is therefore virtually invisible by karyotyping,⁸⁻¹² as confirmed in this study (sensitivity 6%). Karyotyping to detect this translocation should not be considered. The detection of *MLL*

TABLE 4. Results of Diagnostic Accuracy Parameters of Karyotyping, FISH, and RQ-PCR

	Karyotyping	FISH	RQ-PCR
t(9;22)			
Sensitivity (95% CI)	80% (50-100)	100% (100-100)	100% (100-100)
PV – (95% CI)	100% (93-100)	100% (100-100)	100% (100-100)
LR – (95% CI)	0.2 (0.04-1.15)	0 (0-0)	0 (0-0)
t(12;21)			
Sensitivity (95% CI)	6% (0-13)	94% (87-100)	97% (91-100)
PV – (95% CI)	87% (39-100)	98% (93-100)	99% (96-100)
LR – (95% CI)	0.94 (0.87-1.02)	0.06 (0.01-0.21)	0.03 (0.00-0.20)
11q23			
Sensitivity (95% CI)	85% (65-100)	100% (100-100)	30% (2-58)
PV – (95% CI)	99% (94-100)	100% (100-100)	95% (70-100)
LR – (95% CI)	0.15 (0.04-0.55)	0 (0-0)	0.7 (0.47-1.05)

FISH indicates fluorescence in situ hybridization; LR – , likelihood ratio of negative test; PV – , negative predictive value; RQ-PCR, real time quantitative reverse transcriptase polymerase chain reaction; 95% CI, 95% confidence interval.

rearrangements by karyotyping is highly sensitive as shown in this and other studies.^{17,18} Identification can be problematical when the *MLL*-rearrangement is a subtle anomaly and chromosome preparations are of poor quality.^{11,19-21} Even more complicated to detect are small duplications and deletions in the *MLL* region. They are generally not visible by karyotyping.¹⁴ However, the small duplications, will also be difficult to detect by FISH although that method can be used for detection of some of the deletions. For translocations detection, irrespective of the translocation partner, FISH is the method of choice. RQ-PCR is a highly sensitive method, but it only addresses the gene fusion for which it was designed.

FISH analysis is used in many laboratories to confirm the presence of previously characterized chromosome abnormalities or to rule out (suspected) cryptic aberrations, thus compensating the shortcomings of karyotyping. Also, FISH allows large numbers of nondividing cells to be screened. In this study, sensitivity of FISH for detection of *BCR-ABL*, *TEL-AML1* fusions, and *MLL*-rearrangements is very high, which is in concordance to other studies.²²⁻²⁴ FISH seems to be the best diagnostic technique for detecting t(9;22) and t(11q23). For t(12;21) only 2 fusions were missed, which might be due to interpretation or technique failures. FISH using probes for *AML1* and *TEL* does not only provide information on the presence of the t(12;21) itself, but also on the presence of additional abnormalities of chromosome 12 and 21 known to be secondary to the t(12;21), for example deletion of the second *TEL* gene, presence of an additional copy of 21 or der(21) t(12;21).²⁵

In this study, RQ-PCR had a high sensitivity in detecting translocations causing the *BCR-ABL* and *TEL-AML1*, and *MLL-AF4* fusion genes, which is in agreement with other studies.^{26,27} But RQ-PCR was less successful in detecting other *MLL*-rearrangements as it was tested only for t(4;11). The *MLL* gene has multiple potential partner genes and various breaking points, and therefore, rearrangements involving *MLL* are challenging to detect.²²

RQ-PCR analysis only needs small amounts of patient's RNA, it does not require dividing cells and it is extremely sensitive in detecting rare abnormal cells. Furthermore, results are achievable in a short time. A minor point is that a RQ-PCR reaction is specific for only one individual genetic rearrangement and therefore, considering the high number of fusion genes and breakpoint variants characterized, numerous RQ-PCR reactions or a multiplex approach are needed to effectively screen for all ALL-associated rearrangements.^{14,28,29}

To deal with the often limited laboratory and health care resources the question arises whether all 3 diagnostic techniques should be used in detecting t(9;22), t(12;21), and t(11q23). RQ-PCR and FISH seem in some cases redundant, especially if a specific translocation is already detected. Karyotyping is, however, an indispensable tool for discovering numerical, structural, and unexpected chromosomal aberrations. FISH and RQ-PCR have additional value as certain anomalies are cryptic or in those cases where karyotyping has failed. To achieve the highest sensitivity a multimodal approach still optimizes final outcome.

This study has some limitations. First, the results of the diagnostic accuracy determination are clear, but the 95% confidence intervals were often wide. This is the consequence of the small amount of patients with the

specific structural chromosomal translocations involved. Despite the wide intervals, overlap was limited, and therefore, did not influence the interpretation substantially.

Second, not all children were tested with all diagnostic techniques and in some characteristics the tested children differed significantly from the not-tested children. This difference can be explained by the fact that most children who were not tested were diagnosed before 1997. They have worse therapy outcome and their data were difficult to compare, due to alterations in immunophenotyping procedures and detection rate of extramedullary disease. Also changes in proficiency have influenced our data. Until 1999 FISH and molecular genetic analysis was not experienced and standardized in our hospital. In these years chromosomal aberrations such as t(9;22), t(12;21), and t(11q23) could have been missed. Additionally, reporting of especially FISH was restricted in the early years. This could have resulted in incomplete collection of data of these years, subsequently resulting in a slight underestimation in the detection of chromosomal aberrations in the final results of this study. Analyses were carried out to detect changes in sensitivity per anomaly in detection rate, in relation to the period of the investigations (data not shown). Although several techniques tended to increase in detection rate, no significances were noted. This might be due to the limited number of patients.

To overcome difficulties and provide an accurate detection method for chromosomal aberrations, new diagnostic devices, such as multiplex RT-PCR^{16,28,30} with more translocation fusion partners and expression micro arrays^{16,31,32} have been developed and will be introduced in a few years for standard care in most patients. Identification of genetic aberrations and clarification of the resulting molecular pathways will result in both improvement of risk-stratification and application of novel targeted therapies designed to restore normal function of these pathways.

We conclude, that karyotyping, FISH, and RQ-PCR are powerful tools for the detection of the major chromosomal abnormalities in childhood ALL, although each method has its limitation. The complementary use of the techniques in ALL diagnostics, in combination with minimal residual disease detection, will deliver the best available treatment to an individual child.

REFERENCES

1. Coebergh JW, Reedijk AM, de VE, et al. Leukemia incidence and survival in children and adolescents in Europe during 1978-1997. Report from the Automated Childhood Cancer Information System project. *Eur J Cancer*. 2006;42:2019-2036.
2. Secker-Walker LM, Lawler SD, Hardisty RM. Prognostic implications of chromosomal findings in acute lymphoblastic leukemia at diagnosis. *Br Med J*. 1978;2:1529-1530.
3. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006;354:166-178.
4. Rooney D, Czepulkowski B. *Human Chromosome Preparation: Essential Techniques*. Chichester, UK: John Wiley & Sons Ltd; 1997.
5. Schwarzbacher T, Harrison G, Heslop-Harrison P. *Practical In Situ Hybridization*. Abingdon, UK: BIOS Scientific Publishers Ltd; 2000.
6. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. *Leukemia*. 2003;17:2318-2357.

7. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33:159–174.
8. Romana SP, Le Coniat M, Berger R. t(12;21): a new recurrent translocation in acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 1994;9:186–191.
9. Harrison CJ. The genetics of childhood acute lymphoblastic leukemia. *Baillieres Best Pract Res Clin Haematol*. 2000;13:427–439.
10. Douet-Guilbert N, Morel F, Le Bris MJ, et al. A fluorescence in situ hybridization study of TEL-AML1 fusion gene in B-cell acute lymphoblastic leukemia (1984–2001). *Cancer Genet Cytogenet*. 2003;144:143–147.
11. Woo HY, Kim DW, Park H, et al. Molecular cytogenetic analysis of gene rearrangements in childhood acute lymphoblastic leukemia. *J Korean Med Sci*. 2005;20:36–41.
12. Zhang L, Parkhurst JB, Kern WF, et al. Chromosomal changes detected by fluorescence in situ hybridization in patients with acute lymphoblastic leukemia. *Chin Med J (Engl)*. 2003;116:1298–1303.
13. Strehl S, Konig M, Mann G, et al. Multiplex reverse transcriptase-polymerase chain reaction screening in childhood acute myeloblastic leukemia. *Blood*. 2001;97:805–808.
14. Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukemia. *Blood Rev*. 2001;15:49–59.
15. Karhu R, Siitonen S, Tanner M, et al. Genetic aberrations in pediatric acute lymphoblastic leukemia by comparative genomic hybridization. *Cancer Genet Cytogenet*. 1997;95:123–129.
16. Ramos ML, Palacios JJ, Fournier BG, et al. Prognostic value of tumoral ploidy in a series of spanish patients with acute lymphoblastic leukemia. *Cancer Genet Cytogenet*. 2000;122:124–130.
17. Taki T, Ida K, Bessho F, et al. Frequency and clinical significance of the MLL gene rearrangements in infant acute leukemia. *Leukemia*. 1996;10:1303–1307.
18. Borkhardt A, Wuchter C, Viehmann S, et al. Infant acute lymphoblastic leukemia-combined cytogenetic, immunophenotypic and molecular analysis of 77 cases. *Leukemia*. 2002;16:1685–1690.
19. Martin Ramos M, Fernandez Martinez F, Barreiro Miranda E. Cytogenetic abnormalities in acute lymphoblastic leukemia. *An Esp Pediatr*. 2001;55:45–52.
20. Harrison CJ, Moorman AV, Barber KE, et al. Interphase molecular cytogenetic screening for chromosomal abnormalities of prognostic significance in childhood acute lymphoblastic leukemia: a UK Cancer Cytogenetics Group Study. *Br J Haematol*. 2005;129:520–530.
21. Cimino G, Rapanotti MC, Rivolta A, et al. Prognostic relevance of ALL-1 gene rearrangement in infant acute leukemias. *Leukemia*. 1995;9:391–395.
22. Cuthbert G, Thompson K, Breese G, et al. Sensitivity of FISH in detection of MLL translocations. *Genes Chromosomes Cancer*. 2000;29:180–185.
23. Tkachuk DC, Westbrook CA, Andreeff M, et al. Detection of bcr-abl fusion in chronic myelogenous leukemia by in situ hybridization. *Science*. 1990;250:559–562.
24. Martinez-Ramirez A, Urioste M, Contra T, et al. Fluorescence in situ hybridization study of TEL/AML1 fusion and other abnormalities involving TEL and AML1 genes. Correlation with cytogenetic findings and prognostic value in children with acute lymphocytic leukemia. *Haematologica*. 2001;86:1245–1253.
25. Stams WAG, Beverloo HB, den Boer ML, et al. Incidence of additional genetic changes in the TEL and AML1 genes in DCOG and COALL-treated t(12;21)-positive pediatric ALL, and their relation with drug sensitivity and clinical outcome. *Leukemia*. 2006;20:410–416.
26. Cox MC, Maffei L, Buffolino S, et al. A comparative analysis of FISH, RT-PCR, and cytogenetics for the diagnosis of bcr-abl-positive leukemias. *Am J Clin Pathol*. 1998;109:24–31.
27. van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia*. 1999;13:1901–1928.
28. Pallisgaard N, Hokland P, Riishoj DC, et al. Multiplex reverse transcription-polymerase chain reaction for simultaneous screening of 29 translocations and chromosomal aberrations in acute leukemia. *Blood*. 1998;92:574–588.
29. Bench AJ, Erber WN, Scott MA. Molecular genetic analysis of hematological malignancies: I. Acute leukemias and myeloproliferative disorders. *Clin Lab Haematol*. 2005;27:148–171.
30. Salto-Tellez M, Shelat SG, Benoit B, et al. Multiplex RT-PCR for the detection of leukemia-associated translocations: validation and application to routine molecular diagnostic practice. *J Mol Diagn*. 2003;5:231–236.
31. Harrison CJ, Griffiths M, Moorman F, et al. A multicenter evaluation of comprehensive analysis of MLL translocations and fusion gene partners in acute leukemia using the MLL FusionChip device. *Cancer Genet Cytogenet*. 2007;173:17–22.
32. DeRisi J, Penland L, Brown PO, et al. Use of a cDNA microarray to analyze gene expression patterns in human cancer. *Nat Genet*. 1996;14:457–460.