BCR/ABL MOLECULAR TESTING BY FLUORESCENCE IN SITU HYBRIDIZATION IN UNTREATED ADULT ACUTE LEUKEMIA

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Summary. The BCR/ABL detection influences diagnosis, prognosis and treatment decisions in patients with chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). We studied the feasibility and the sensitivity of LSI BCR/ABL dual-colour, dual-fusion probe as a usual assay for detection BCR-ABL gene fusion in acute leukemia. A total of 3229 successfully hybridized interphase nuclei from 34 newly diagnosed patients with acute leukemias (12 AML and 22 ALL cases) were analyzed. We used interphase fluorescence in situ hybridization (FISH) method and LSI BCR/ABL dual-colour, dual-fusion probe (Vysis) for detection of BCR-ABL gene that span the common breakpoints of t(9;22)(q34;q11.2) and that detect double BCR-ABL fusion in bone marrow cells with this translocation, one on the abnormal chromosome 9 and one on the Philadelphia chromosome (Ph chromosome). Fusion BCR-ABL gene was observed in 9 (28.13%) of the cases with successful hybridization and in 6 (18.75%) of them the results had clinical reliability. The aberrant cell lines were of different sizes – 53%-96% of cells were positive for BCR-ABL gene. Fusion signals for BCR-ABL were reported in 2 (16.67%) of AML and in 4 (18.18%) of ALL cases. In conclusion, we proved that LSI BCR-ABL dual-colour, dual-fusion probe (Visys) is characterized with high specificity and sensibility.

Key words: *Interphase FISH, BCR-ABL gene, adult acute leukemia*

INTODUCTION

BCR-ABL, a common molecular defect in 95% of patients with chronic myeloid leukemia (CML), in 20-30% of adult acute lymphoblastic leukemia (ALL) and in 1-2% of adult acute myeloid leukemia (AML) [2,

15] is a valuable tumor marker whose detection influences prognosis and clinical management decisions.

The diagnosis of the BCR/ABL fusion gene in acute adult leukemias has important clinical implications because it is the most common molecular genetic change in adult ALL and is associated with short remissions and poor outcome in all age groups [2, 9, 10, 13]. The BCR-ABL fusion gene arises as a result of a reciprocal translocation between chromosomes 9 and 22, resulting in the Philadelphia (Ph) chromosome. A variable sequence length of the BCR gene at 22q11 fuses with ABL at 9q34 and encodes the constitutively active BCR-ABL protein tyrosine kinase [1].

Conventional cytogenetics is the recommended test for detecting t(9;22) in newly diagnosed leukemia patients [10, 17]. Chromosome banding analysis has the advantage of high specificity and an ability to detect alternate or additional cytogenetic changes that influence the diagnosis and prognosis. Diagnosis of the BCR/ABL fusion in ALL is difficult because the molecular findings are more heterogeneous than they are in CML [15, 16, 17]. In ALL, karyotype analysis using banding techniques is unsuccessful in 10% to 20% of cases due to bad chromosome morphology or a lack of metaphase. In another 30% to 40% of ALL cases, only normal metaphases are found. In these cases, ALL blasts may not have been investigated.

Molecular methods such as fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chain reaction (rtPCR), and real-time quantitative rtPCR can be used to detect the chimeric BCR-ABL gene or its transcripts.

The advantages of FISH over G-banding include applicability to interphase cells, greater sensitivity (as many more cells can be analyzed), and ability to detect cryptic (about 5% of CML and small proportion of ALL cases) and complex translocations [11, 14, 16]. This has led to its use in the clinical setting for monitoring the response to therapy, by quantifying the size of the BCR-ABL (+) cell lines.

AIM OF THE STUDY

The aim of the study was to evaluate the reliability and specificity of FISH method and LSI BCR/ABL dual-colour, dual-fusion probe for the detection of the Ph translocation in acute leukemia.

MATERIAL AND METHODS

Design of the study

A total of 3229 successfully hybridized interphase nuclei from 34 untreated adult patients with ALL (22 cases) and AML (12 cases) were analyzed. FISH testing was performed in 5 patients with classical t(9;22)(q34;q11.2), in 8 patients with unsuccessful cytogenetic analyses and in 21 cases with other cytogenetic changes for detection of concealed BCR-ABL fusion genes.

Methods

Conventional cytogenetics

Cytogenetic analyses were performed on short term (24 ± 48 h) bone marrow cultures according to standard procedures. Chromosomes were identified using G-banding technique and classified according to the International System for Human Cytogenetic Nomenclature (ISCN) [8]. At least 15 metaphases were analyzed for each case.

Interphase fluorescence in situ hybridization (FISH)

For interphase FISH we used a fixed cell nuclei by conventional cytogenetic procedures or native bone marrow smears. In each case at least 100 successfully hybridized interphase nuclei were analyzed.

We used LSI BCR/ABL dual-colour, dual-fusion probe (Vysis) that span the common breakpoints of t(9;22)(q34;q11.2) and that detect double BCR/ABL fusion in cells – one on the abnormal chromosome 9 and one on the Philadelphia chromosome (Ph+). The LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe is a mixture of the LSI BCR probe labeled with SpectrumGreen and the LSI ABL probe labeled with SpectrumOrange. The spanning ABL probe has a genomic target of approximately 650 kb extending from an area centromeric of the argininosuccinate synthetase gene (ASS) to well telomeric of the last ABL exon. The BCR probe target spans a genomic distance of about 1.5 Mb. The BCR probe begins within the variable segments of the immunoglobulin lambda light chain locus (IGLV), extends along chromosome 22 through the BCR gene, and ends at a point approximately 900 kb telomeric of BCR (Fig. 1).

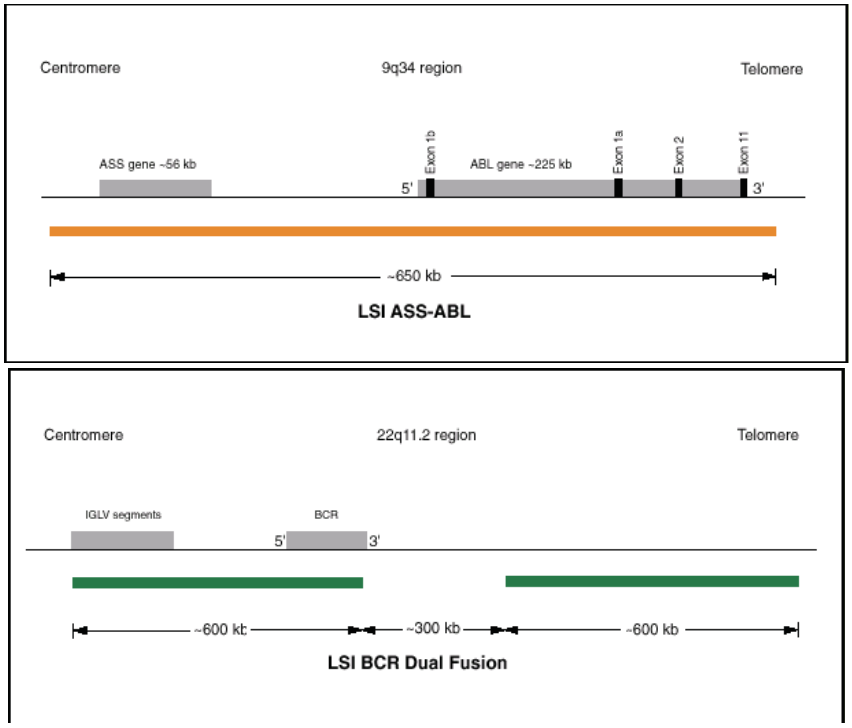


Fig. 1. Map of DNA regions on chromosomes 9 and 22, detectible by LSI BCR/ABL dual-colour, dual-fusion probe (Vysis)

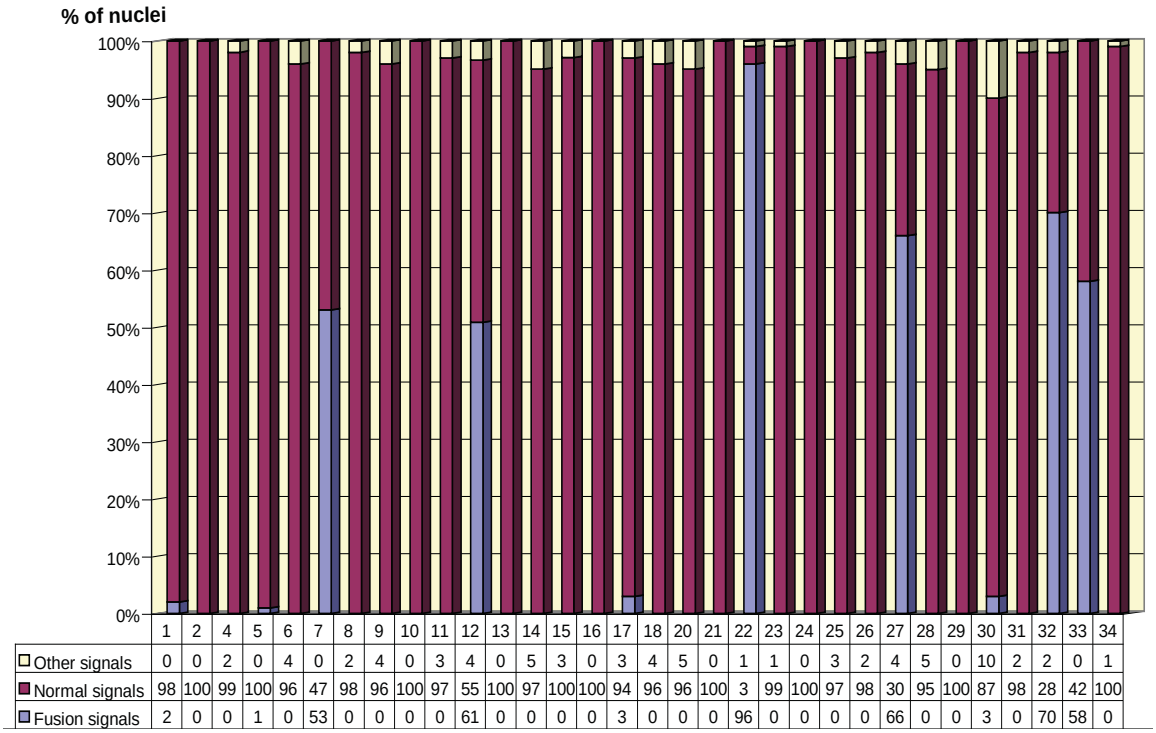
Bone marrow cells were denaturized with 70% deionised formamide/2 x SSC for 5 minutes at 70°C. We placed 10 µl hybridization solutions (7 µl buffer, 1 µl labeled DNA probe and 2 µl aqua destillata) on the cell containing area of the slides, covered it with a cover glass, and sealed with rubber cement. In situ hybridization was performed at 370C for 16 hours in a humidified chamber. Posthybridization washes were performed in a 0.4 x SSC solution at 72°C for 2 minutes and transferred in 4xSSC/1% “Tween-20”solutions. Chromosomes were counterstained with 12 µl (for 24 x 24 mm cover glass) DAPI/Antifade. Fluorescent signals were viewed with an OLYMPUS fluorescence microscope equipped with a computer software ISIS imaging system (MetaSystems, Germany).

After hybridization two orange and two green signals (2O2G) were observed in normal cell nuclei. In the cases with BCR/ABL fusion gene two fusion orange/green (yellow), one green and one orange signals (1O1G2F) were observed.

RESULTS

Conventional cytogenetic analysis was successfully performed in 24 of 34 cases (70.58%) and a t(9;22) was identified in 5 cases (20.83%). Seven cases had a normal karyotype, and 12 had chromosome abnormalities without Ph translocation and 3 carried chromosome abnormalities in combination with Ph translocation.

The results of FISH studies are summarized in Figure 2.



	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Other signals	0	0	2	0	4	0	2	4	0	3	4	0	5	3	0	3	4	5	0	1	1	0	3	2	4	5	0	10	2	2	0	1
Normal signals	98	100	99	100	96	47	98	96	100	97	55	100	97	100	100	94	96	96	100	3	99	100	97	98	30	95	100	87	98	28	42	100
Fusion signals	2	0	0	1	0	53	0	0	0	0	61	0	0	0	0	3	0	0	0	96	0	0	0	0	66	0	0	3	0	70	58	0

Fig. 2. Results, obtained by dual-color, dual-fusion hybridization in 12 AML (1-12) and 22 ALL (12-24) patients

Fusion BCR/ABL gene was found in 9 (28.13%) of successfully hybridizated nuclei, as in 6 (18.75%) of them the findings had clinical reliability (Fig. 2, Fig. 3). The percentage of leukemic cells with two fusion signals ranged from 58% to 96% (mean 72.5%) for ALL and from 53% to 61% (mean 57%) for AML cases. BCR/ABL fusion positive signals were recorded in 2 (16.67%) of all AML cases and in 4 (18.18%) of ALL cases.

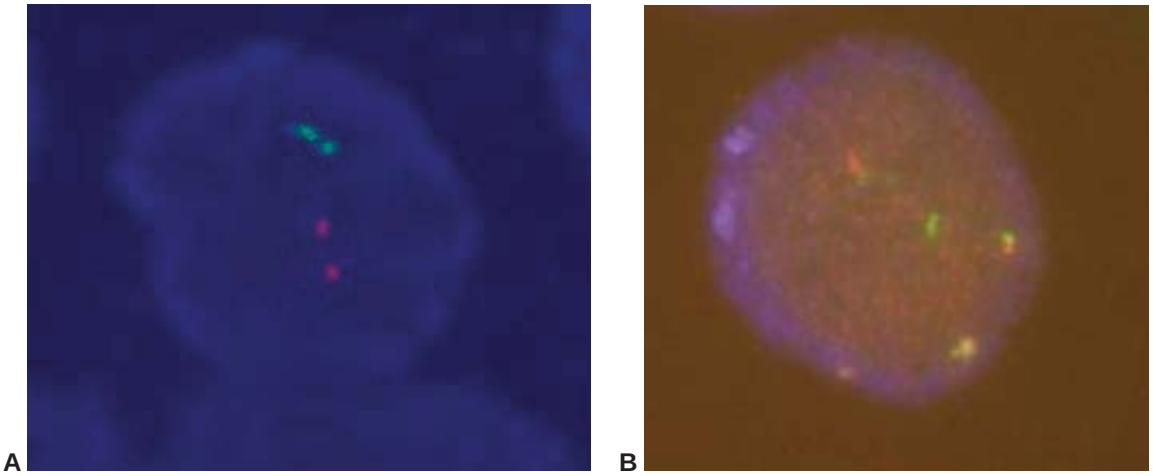


Fig. 3. A: LSI BCR/ABL dual-colour, dual-fusion translocation probe (Vysis) hybridized to a nucleus without t(9;22). Two orange and two green signals were observed; **B:** LSI BCR/ABL dual-colour, dual-fusion translocation probe (Vysis) hybridized to a nucleus containing a simple balanced t(9;22). One orange, one green and two orange/green (yellow) fusion signals are observed

Single interphase nuclei (1 to 3 nuclei of 100 cells analyzed) with a positive gene fusion were observed in 3 patients and were considered sporadic and occasional fluorescent artifacts (Fig. 2).

The percentage of cells with t(9;22) or BCR/ABL fusions, detected by both methods, are demonstrated in Table 1.

Table 1. Comparative analysis of t(9;22)-BCR/ABL frequency, detected by two methods – conventional cytogenetics and FISH

N from tabl.1	Type of acute leukemia	% Clonality of t(9;22) (Conventional cytogenetics)	% Clonality of BCR/ABL fusion gene (FISH)
7	AML-M1	t(9;22)(q34;q11)- 33.3%	Bcr/abl- 53%
12	AML-M1	t(9;22)(q34;q11) - 27.3%	Bcr/abl- 61%
22	ALL	unsuccessful	Bcr/abl- 96%
27	ALL	t(9;22(q34;q11) - 46.7%	Bcr/abl- 66%
32	ALL	t(9;22(q34;q11)- 54.5%	Bcr/abl-70%
33	ALL	t(9;22(q34;q11)- 55%	Bcr/abl- 58%

DISCUSSION

The efficacy of interphase FISH analysis for the identification of BCR/ABL rearrangement in CML patients is well documented [3, 6, 9, 12]. However, in adult ALL the role of this technique for the detection of Ph+ patients has been tested only in some series [2, 6, 9, 10] and the authors have used primarily dual color probes detecting the BCR/ABL rearrangement as a single fusion signal. This kind of probes limits the specificity of interphase FISH, because the BCR and ABL signals may coincidentally overlap in rates ranging between 4 and 10% of the nuclei [9]. This technical artifact may give a false interphase FISH diagnosis of Ph+ acute leukemia.

In the present study we tested the feasibility and efficacy of dual color/dual fusion FISH probe in patients with acute leukemias. The molecular equivalent of t(9;22) – BCR/ABL fusion gene was studied in most ALL patients and in 12 AML patients to demonstrate the cytogenetic finding or in connection with unsuccessful cytogenetic analysis. We detected two well visible fusion signals in all of Ph+ positive nuclei, without overlapping or missing (Fig 3B). In the Ph negative interphase nuclei and metaphases, we recorded 2 green (from normal 22q11.2 region) and 2 red (from normal 9q34 region) fluorescent signals (Fig. 3A).

The incorrect scoring of a normal nucleus as abnormal was rare due to the possible detection of two fusion signals by BCR/ABL dual-colour, dual-fusion FISH probe [7]. The probe was powered to detect a BCR/ABL rearrangement in all cases with t(9;22) by conventional cytogenetics, including 2 AML-M1 cases with t(9;22).

In some cases, single interphase nuclei (less than 3%) had 1 or 2 positive gene fusion signals. According to guidelines for FISH application [16], this is a very low percent of positive cells in patients with acute leukemia and we considered them as sporadic and occasional fluorescent artifacts.

CONCLUSION

LSI BCR/ABL dual-colour, dual-fusion probe (Visys) for detection of BCR-ABL gene is characterized by high specificity and sensitivity. By the proof of fusion of two signals excluding the possibility of misinterpretation of close together marked in interphase cell genes. Through this FISH probe we can identify masked or undetected by conventional cytogenetics BCR-ABL translocations.

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