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Applications of Fluorescence in Situ Hybridization (FISH) for Detecting Genetic Changes in Hematological Malignancies

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ABSTRACT

Fluorescence in situ hybridization (*FISH*) *has become an important tool both for defining initial chromosomal abnormalities within a disease process*, *and for monitoring response to therapy as well as minimal residual disease. We report the results of interphase FISH* (*iFISH*) *analysis of* 92 *patients. We have used five different FISH probes to detect common cytogenetic rearrangements associated with hematological malignancies. A total of* 83 *patients were screened for BCR/ABL gene rearrangements. Displayed iFISH patterns of BCR/ABL gene rearrangements in* 37.3% *of patients* (31/83) *ranged between* 10% *to* 98%. *In addition*, *while* 3 *patients and one patient with AML showed t*(15; 17) (12.5%) *and inv*(16; 16) (8.3%) *respectively*, *t*(8; 21) *was not found. Furthermore*, *secondary chromosomal aberrations* (6.5% *of all cases*) *were clearly non random in the present study. The diagnosis of BCR*/*ABL gene rearrangements are likely become an important tool for the monitoring of therapies in patients with CML. Atypical patterns also may have clinical prognostic implications. Further studies in larger groups of patients are needed in order to elucidate the role of AML1/ETO*, *PML/RARA*, *CBFB and p*53, *and to identify the specific chromosomal regions and interacting genes involved in this process.*

***Keywords*:** *Flourescence in Situ Hybridization*, *BCR/ABL*, *Gene Rearrangements*

1. Introduction

The recent World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues emphasizes the importance of chromosomal abnormalities for accurate diagnosis, appropriate treatment, and monitoring response to therapy [1]. Human malignancies may be developed by a variety of mechanisms, including inactivation of tumor suppressor genes, activation of oncogenes, and general genomic instability. Tumor-specific chromosomal translocations, and other genetic abnormalities, have been described for a large number of hematopoietic and lymphoid malignancies [2,3]. For many years, conventional karyotyping has been used as the sole diagnostic tool for hematologic malignancies. Flouresence In Situ Hybridization (FISH) provides an important adjunct to conventional cytogenetics and molecular studies in the evaluation of chromosome abnormalities associated with hematologic malignancies. In addition, FISH analysis offers one of the most sensitive, specific, and reliable strategies for identifying acquired genetic abnormalities such as characteristic gene fusions, aneuploidy, loss of a chromosomal region associated with hematologic disorders, and serving as a technique that can help in both the diagnosis of a genetic disease or suggesting prognostic outcomes. It is especially important for cells of patients with leukemia, where the quality of metaphases is often not so good, and is frequently used to monitor the response to therapy in various hematological malignancies [4]. Thus, FISH is widely used today in clinical practice to help in diagnosis and selects appropriate treatments for patients with hematological malignancies [5]. In research, FISH studies are used to investigate the origin and progression of hematological malignancies, and to establish which hematopoetic compartments are involved in neoplastic processes [6]. Interphase FISH (iFSH) is increasingly used for the identification of *BCR/ABL* gene rearrangements in CML, AML, ALL and CLL. The aim of this study was to

determine the frequency of FISH patterns of *BCR*/*ABL*, *AML*1/*ETO*, *PML*/*RARA*, and *p*53 genes as a diagnostic tool to understand the pathophysiology, diagnosis, treatment, prognosis, and monitoring of disease activity in CML, AML, ALL, CLL and MDS cases.

2. Materials and Metods

2.1. Patients

A total of 92 patients with a known hematological disorder including 50 (54.34%) CML, 25 (27.2%) AML, 7 (7.6%) ALL, 4 (4.35%) CLL, and 6 (6.52%) MDS patients diagnosed at Departments of Hematology were referred to Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University for a routine iFISH analysis. There were 59 males and 33 females. Their ages ranged from 20 to 81 years, with a mean (±SD) age of 50, 45 ± 15, 19 years (**Tables 1** and **2**).

2.2. Slide Preparation and Flourescence *in Situ* Hybridization

A 2-ml venous blood taken from all patients in order to determine the t(9; 22), t(8; 21), t(15; 17), and/or inv(16) anomalies, and/or deletion of *p*53 gene. Standard techniques were used for harvesting and slide preparation without incubation. After incubating slides at room temperature overnight, fluorescence *in situ* hybridization was performed. For these purposes, LSI *BCR*/*ABL* ES Dual Color Translocation probe (Vysis), LSI *PML*/*RARA* Dual Color Dual Fusion Translocation Probe (Vysis), LSI *AML*1/*ETO* Dual Color Dual Fusion Translocation Probe (Vysis), LSI *CBFB* Dual Color Break Apart Rearrangement Probe (Vysis) and LSI *p*53, 17p13.1, Spectrum Orange Probe (Vysis) were used. Firstly slides were pretreated with 2XSSC for 5 min at room temperature and then immersed them in the solution contained HCl (1N), water and pepsin A (2:200:2 v/v/v) for 30 min at 37˚C. After the time, slides were washed with water immediately. Then, they were washed with PBS, PBS/$MgCl_2{\cdot}6H_2O$, and PBS/$MgCl_2{\cdot}6H_2O$ with paraformaldehyde for 2 min, 2 min, and 10 min, respectively, and then passed through a dehydration series of 70, 85 and 100% ethanol for 3 min each. Then slides were left to dry. Simultaneously, 10 µl of each probe mixtures were applicated on slides immediately, and a coverslip was sealed onto the slides with rubber cement. The slides put in ThermoBrite Denaturation/Hybridization System and denaturated 5 min at 95˚C and hybridized overnight at 37˚C. For posthybridization process, slides were washed with 0.4XSSC/0.3% Tween 20 for 2 min at 73˚C and 2XSSC/0.1% Tween 20 for 1 min at room temperature, respectively. After the slides were waited for drying at a dark room. In the next step, DAPI tube was vortexed and slides were counterstained with 10 µl of it, and then waited 30 min at –20˚C. At the end of time, slides were analysed at flourescent microscopy using red, green and DAPI filters. Interphase cells were analyzed using a BX51 Olympus fluorescence microscope equipped with Cytovision Probe Software (Applied Imaging, Santa Clara, CA). For each case and probe, a minimum of 100 interphase cells was evaluated for the signal patterns.

3. Results

In the present study, 92 hematological patients including CML [50 (54.34%)], AML [25 (27.17%)], ALL [7 (7.6%)], CLL [4 (4.35%)], and MDS [6 (6.52%)] were analyzed for some parameters [t(9; 22), t(8; 21), t(15; 17), inv (16; 16) and *p*53] (**Table 1**).

A total of 83 patients were screened for *BCR/ABL* gene rearrangements. The great majority of the patients analyzed including most CML (25/48, 52.1%), AML (4/22, 18.2%), ALL (1/7, 14.3%) and MDS (1/6, 16.7%) cases displayed iFISH patterns of *BCR/ABL* gene rearrangements (31/83, 37.3%) ranged between 10% to 98% (**Tables 2** and **3**). Fifty-two percent of CML patients demonstrated Ph translocation while 48% were negative for the Ph chromosome. Approximately 96.2% of Ph-positive patients displayed the typical FISH signal pattern. The iFISH patterns of *BCR*/*ABL* gene rearrangements showed four patterns. Pattern A, typical iFISH pattern, consisted of one fusion-der (22)-, one greennonrearranged 22- and two red-der (9) plus the nonrearranged chromosome 9- signals (1F2R1G) (29/83, 34.9%). Atypical patterns (1F1R1G and1F1R2G) among the Ph-positive patients included atypical *BCR*/ *ABL* fused gene rearrangements on chromosome 9 or 9q deletion of the rearranged chromosome 9; coexistence of der(9q) and der(22q) deletions (2/25, 7.7%) (**Table 4**) (**Figure 1**).

There are 27 patients- 23 CML, 2 AML, 1 ALL and one MDS- showed typical iFISH pattern (Pattern A) (**Table 3**, **Figure 1**). On the other hand, as illustrated in **Figure 1** and shown in **Table 4**, pattern B (one fusion, one red and one green signals) was found in 3 patients (3.6%) included 1 CML and 2 AML. Pattern C (one fusion, one red and two green signals) was shown by

Table 1. The distribution of hematological cancers in the present study.

Hematological disorder	n (%)
Chronic myeloid leukemia (CML)	50 (54.34)
Acute myeloid leukemia (AML)	25 (27.17)
Acute lymphoblastic leukemia (ALL)	7 (7.6)
Chronic lymphoblastic leukemia (CLL)	4 (4.35)
Myelodysplastic syndrome (MDS)	6 (6.52)
Total	**92**

Table 2. The demographic information and iFISH results of the study population.

Case No	Age	Sex	Disease	*BCR/ABL* [t(9; 22)] %	*AML1/ETO* [t(8; 21)] %	*PML/RARA* [t(15; 17)] %	*CBFB* [inv(16; 16)] %	*P53* [del(p13.1)] %	The other chromosomal aberrations (%)
C1	42	M	CML	**10**	−	−	−	−	−
C2	64	M	CML	**21**	−	−	−	−	−
C3	49	F	CML	**77**	−	−	−	−	−
C4	38	F	CML	**12**	−	−	−	−	−
C5	57	M	CML	**50**	−	−	−	−	−
C6	70	F	AML	−	−	0	−	−	−
C7	58	F	CML	**80**	−	−	−	−	−
C8	64	M	CML	0	−	−	−	−	−
C9	62	M	CML	**84**	−	−	−	−	−
C10	44	F	CML	0	−	−	−	−	−
C11	70	M	AML	3	7	4	**12**	−	−
C12	49	M	CML	**86**	5	9	5	−	−
C13	58	F	CML	7	5	5	8	−	−
C14	77	M	MDS	**11**	2	3	5	−	−
C15	70	M	AML	0	3	3	8	−	−
C16	68	F	ALL	6	3	6	3	−	−
C17	45	M	CML	**88**	−	−	−	−	−
C18	29	M	CML	**88**	−	−	−	−	−
C19	46	F	CML	**37**	−	−	−	−	−
C20	30	M	CML	**26**	−	−	−	−	−
C21	37	M	CML	**89**	2	5	1	−	−
C22	44	F	ALL	5	2	7	5	−	−
C23	70	M	CML	0	−	−	−	−	−
C24	41	M	CML	0	−	−	−	−	−
C25	37	F	AML	8	2	4	1	−	−
C26	35	F	AML	−	2	5	2	−	−
C27	43	M	AML	**12**	8	5	3	−	−
C28	44	F	AML	**55**	2	**11**	3	−	−
C29	52	M	AML	**10**	2	4	2	−	−
C30	59	M	ALL	**21**	−	−	−	−	−
C31	81	F	CML	6	1	6	0	−	−
C32	38	F	CML	**97**	−	−	−	−	−
C33	39	F	MDS	9	0	3	−	−	−
C34	36	M	ALL	6	4	4	−	−	−
C35	74	F	AML	5	3	8	−	−	**Monosomy 9 (70%)**
C36	21	M	AML	**11**	−	9	−	−	−
C37	75	M	AML	6	5	9	−	−	−
C38	54	M	MDS	5	1	6	−	−	**Trisomy 8 (16%)**
C39	70	F	CML	6	−	−	−	−	−
C40	20	F	CML	0	2	0	−	−	−
C41	60	M	CML	**86**	0	0	−	−	−
C42	77	M	CML	0	−	−	−	−	−
C43	59	M	MDS	7	−	−	−	−	−
C44	51	M	CML	**88**	−	−	−	−	−
C45	60	F	CML	**93**	−	−	−	−	−
C46	46	M	CLL	−	−	−	−	−	−
C47	55	M	AML	2	1	**79**	−	−	−
C48	70	M	MDS	0	0	1	−	−	−
C49	61	F	AML	3	0	5	−	−	**Monosomy 17 (25%) Trisomy 8 and monosomy 21 (37%)**
C50	76	M	CML	8	−	−	−	−	−
C51	56	M	CLL	−	−	−	−	0	−
C52	70	F	CML	1	−	−	−	−	−
C53	70	F	AML	2	−	−	−	0	−
C54	60	M	CML	−	−	−	−	**10**	−
C55	63	F	CML	**96**	−	−	−	−	−

C56	56	M	CML	6	–	–	–	–	–
C57	56	M	CML	5	–	–	–	–	–
C58	43	M	CML	3	–	–	–	–	–
C59	59	M	CML	**86**	–	–	–	–	–
C60	69	M	CML	7	–	–	–	–	–
C61	51	M	CML	–	–	–	–	0	–
C62	23	F	ALL	7	–	–	–	–	–
C63	40	M	AML	6	–	2	–	–	–
C64	35	F	MDS	6	0	4	–	–	–
C65	53	M	AML	0	0	8	0	–	–
C66	69	F	AML	–	1	4	2	–	**Trisomy 21 (85%)**
C67	35	F	AML	4	-	**86**	0	–	**Trisomy 8 (3%)**
C68	33	M	AML	3	4	5	–	–	–
C69	54	M	AML	2	-	6	0	–	–
C70	54	M	AML	0	0	3	–	–	–
C71	47	M	AML	9	0	4	–	–	**Trisomy 8 (98%)**
C72	64	M	AML	4	0	4	–	–	–
C73	42	M	AML	0	–	0	0	–	–
C74	30	F	CML	**85**	–	–		–	–
C75	54	M	CML	1	0	3	–	–	–
C76	38	F	ALL	4	0	0	–	–	**Tetrasomies 8 and 21 (55%)**
C77	48	M	AML	5	4	0	–	–	**Trisomy 8 (89%)**
C78	23	M	CML	4	–	–	–	–	–
C79	46	F	CML	0	–	–	–	–	–
C80	60	M	CLL	–	–	–	–	0	–
C81	49	M	CML	**45**	–	–	–	–	–
C82	58	M	CML	**93**	–	–	–	–	–
C83	54	F	CML	6	–	–	–	–	–
C84	25	F	CML	5	–	–	–	–	–
C85	47	M	CML	8	–	–	–	–	–
C86	46	M	CLL	–	–	–	–	4	–
C87	28	M	CML	**20**	–	–	–	–	–
C88	41	M	CML	5	–	–	–	–	–
C89	26	M	CML	**90**	–	–	–	–	–
C90	25	M	CML	**98**	–	–	–	–	–
C91	22	F	ALL	3	0	4	–	–	–
C92	44	M	CML	0	–	–	–	–	–

Table 3. The distribution of the patients according to the results of BCR/ABL, AML/ETO, PML/RARA, CBFB and *P*53.

	% (Positive results/total number of patients)				
Hematologic disorder	*BCR/ABL* [t(9; 22)]	*AML/ETO* [t(8; 21)]	*PML/RARA* [t(15; 17)]	*CBFB* [inv(16; 16)]	*p*53 [del(p13.1)]
CML	**52.1 (25/48)**	0 (0/7)	0 (0/7)	0 (0/4)	**50 (1/2)**
AML	**18.2 (4/22)**	0 (0/18)	**12.5 (3/24)**	**8.3 (1/12)**	0 (0/1)
ALL	**14.3 (1/7)**	0 (0/5)	0 (0/5)	0 (0/2)	–
MDS	**16.7 (1/6)**	0 (0/5)	0 (0/5)	0 (0/1)	–
CLL	–	–	–	–	0 (0/4)
Total	**83**	**35**	**41**	**19**	**7**

Table 4. Distribution of typical and atypical iFISH patterns with the ES probe in BCR/ABL+ leukemias studied at diagnosis.

iFISH pattern with *BCR/ABL* ES probe	Chromosomal localization of signals			Number of Ph positive cases (%)			
	F	R	G	CML (n = 25)	AML (n = 4)	ALL (n = 1)	MDS (n = 1)
A: 1F 2R 1G	1F(Ph)	2R(9)	1G(22)	**23 (92%)**	**2 (50%)**	1 (100%)	1 (100%)
B: 1F 1R 1G	1F(Ph)	1R(9)	1G(22)	1 (4%)	**2 (50%)**	–	–
C: 1F 1R 2G	1F(Ph)	1R(9)	2G(22,22)	1 (4%)	–	–	–

F: fusion, R: red, G: green, A: Representative schemes of nuclei carrying typical *BCR/ABL*; B and C: atypical *BCR/ABL* fused gene rearrangements on chromosome 9 or 9q deletion of the rearranged chromosome 9; coexistence of der(9q) and der(22q) deletions.

only one CML patients (2%) (**Table 4**, **Figure 1**). Although t(8;21) was not detected in our patients, the t(15;17) was seen in three AML patients [3/41 (7.31%) analyzed patients for *PML/RARA*], C28, C47, and C67, with the rates of 11%, 79%, and 86%, respectively (**Tables 2** and **3**, **Figures 2(a)** and **(b)**). Invertion (16; 16) was studied in 19 patients. Only one AML patient (C11) showed this invertion in 12/100 interphase cells analyzed (**Tables 2** and **3**, **Figures 2(c)** and **(d)**). The *p*53 gene deletion were screened in 7 patients included 2 CML, one AML, and 4 CLL. Only one CML patient (C54) showed positive result with 10% percentage. The other patients didn't show any deletion in *p*53 gene (**Tables 2** and **3**).

On the other hand, some numerical chromosomal deficiencies or gains were observed in 8/92 of our patients. In C35, a 74-years old AML patient, monosomy 9 was seen in 70/100 analyzed cells. In C38 (MDS), C67 (AML), C71 (AML), and C77 (AML), trisomy 8 cells were obtained in 16%, 3%, 98%, and 89%, respectively. Also, trisomy 21 in C66 (AML) (85/100 cells), tetrasomies 8 and 21 in C76 (ALL) (55%) and, trisomy 8 together with monosomy 21 (37%) and monosomy 17 (25%) in C49 (AML) were seen in the study (**Table 2**, **Figure 3**).

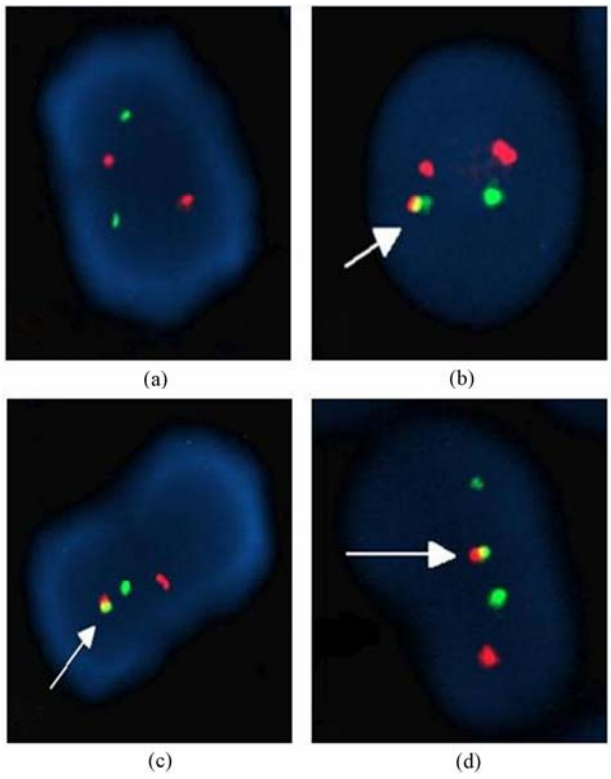

Figure 1. Different interphase FISH (iFISH) patterns found with the LSI *BCR/ABL* ES Dual Color Translocation probe (a) Normal nuclei, (b) 1F 1G 2R pattern (pattern A), (c) 1F 1G 1R pattern (pattern B), (d) 1F 2G 1R pattern (pattern C).

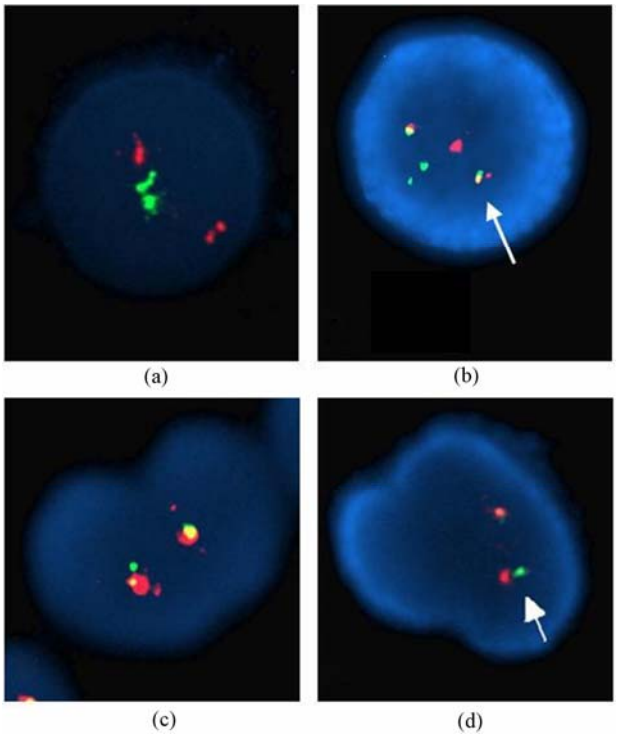

Figure 2. Interphase nuclei showing normal pattern and t(15;17) found with LSI *PML/RARA* Dual Color Dual Fusion Translocation Probe (a-b), normal pattern and inv(16) found with LSI *CBFB* Dual Color Break Apart Rearrangement Probe (c-d).

4. Discussion

In this study, we report the results of *BCR*/*ABL*, *AML*1/*ETO*, *PML*/*RARA*, *CBFB* and *p*53 gene rearrangements in hematological disorders including CML, AML, ALL, CLL and MDS as a genetic and molecular diagnostic model and try to understand their role in the pathophysiology, prognosis, and monitoring of these disease processes.

CML is the first hematological cancer type known to be associated with a specific clonal expansion of t(9; 22). All patients with CML have an abnormal clone with fusion of *BCR* and *ABL*1 loci; at least 90% of patients have a t(9; 22), and the rest have a complex or cryptic variant of this translocation. This finding was first reported in CML cases by Hagemeier *et al.* [7] and afterwards by other authors [8-11]. Our results show that among cases studied at diagnosis, the presence of positive *BCR/ABL* fusion results were more frequently observed in CML (52.1%) compared to AML (18.2%), ALL (14.3%) and MDS (16.7%) cases (**Table 3**). Among Chinese patients with CML, the 62% of 158 cases were Ph positive [12]. In another report, 87% of CML patients were positive for the Ph chromosome [13].

We detected a *BCR*/*ABL1* fusion in 14.3% of adults

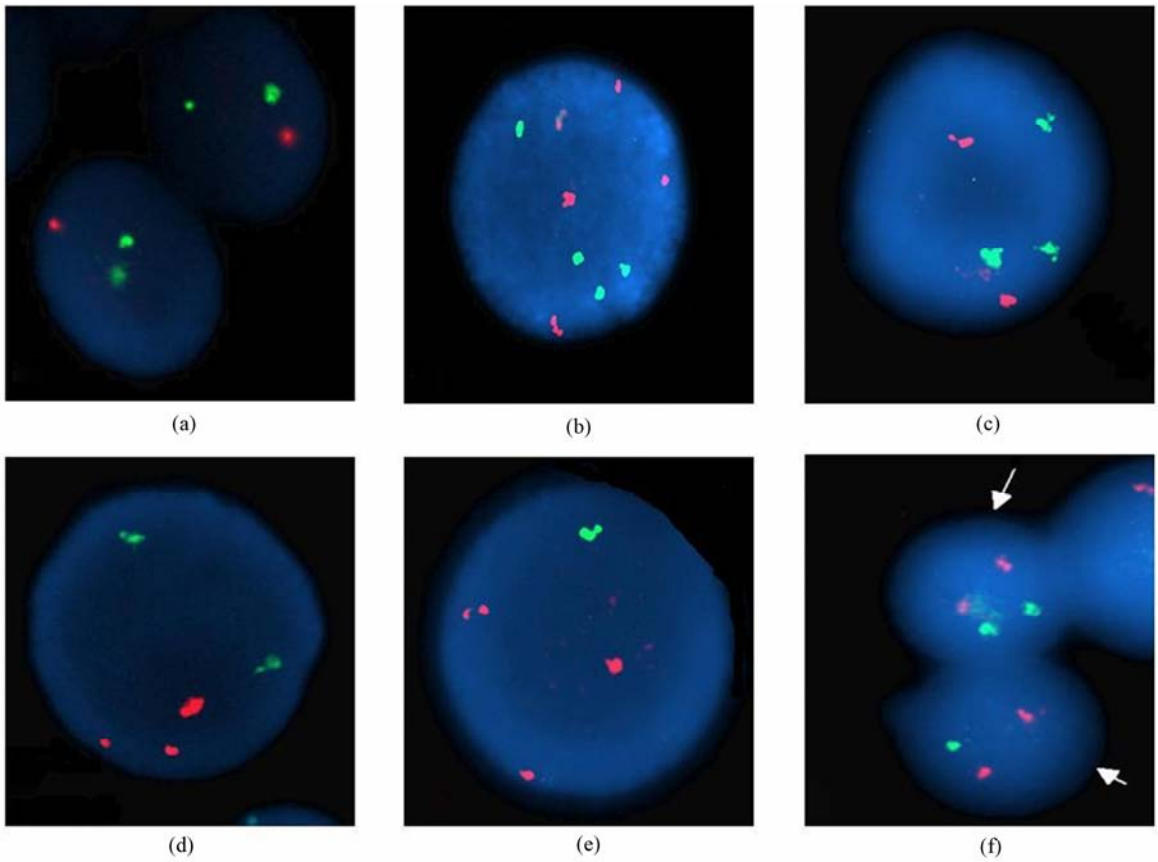

Figure 3. Abnormal FISH patterns (a) monosomy 9 with LSI *BCR/ABL* ES Dual Color Translocation probe, (b) tetrasomies 8 and 21, (c) trisomy 21, (d) trisomy 8, (e) trisomy 8 and monosomy 21 found with LSI *AML1/ETO* Dual Color Dual Fusion Translocation Probe, (f) del (17) found with LSI *PML/RARA* Dual Color Dual Fusion Translocation Probe.

with ALL and 18.2% of adults with AML. A similar *BCR*/*ABL1* fusion occurs in 17% of adults with ALL and 1% of patients with AML [14]. Some investigators have suggested that these Ph-positive forms of acute leukemia may be similar to lymphoid or myeloid blast crisis of CML [15]. Such findings emphasize the importance of performing baseline FISH studies and that these patterns are verified using metaphases since the signal patterns may change during the course of the disease. Among the diagnostic studies, the use of *BCR*/*ABL* FISH ES probe was of more benefit in patients with CML among suspected hematologic malignancies and may confer a worse prognosis. Further studies in larger groups of patients are needed in order to elucidate the role of *AML*1/*ETO*, *PML*/*RARA*, *CBFB* and *p*53, and to identify the specific chromosomal regions and interacting genes involved in this process.

The establishment of signal patterns with FISH is important as atypical patterns may have clinical diagnostic and prognostic implications. Additional karyotypic changes may occur in *BCR/ABL*+ CML, ALL and AML cases [16-18]. These atypical iFISH patterns were most frequently seen as a result of additional numerical changes (most often gain or loss of chromosome 9 or 22). In the present study, the frequency (37.3%) of both typical and atypical *BCR*/*ABL* gene rearrangements was analyzed in 83 patients with CML, AML, ALL and MDS. The most frequently detected patterns with the ES probe corresponded to typical *BCR/ABL* gene rearrangement involving the 1F2R1G (one fusion, two red and one green signals). Among the 48 CML cases, 25 cases were Ph positive, of which 23 cases (92%) were typical FISH pattern (1F2R1G), the other 2 cases (8%) showed 2 different types of atypical FISH patterns (1F1R1G and 1F1R2G) (**Table 4**, **Figure 1**). Both patterns 1F1R1G and 1F1R2G were not found in ALL and MDS cases, but observed in CML and AML cases. In a study including Chinese patients with CML, Philadelphia positive cases (70.4%) demonstrated typical FISH pattern; 29.6% had atypical FISH pattern with 12 different types [12]. The frequency of atypical FISH patterns is comparable to the 15% reported by Cohen *et al.* [19] and Reid *et al.* [20]. In our study, 8% of patients with CML had atypical FISH patterns that the atypical FISH patterns were due to

variant translocation; atypical BCR/ABL fused gene rearrangements on chromosome 9 or 9q deletion of the rearranged chromosome 9; coexistence of der(9q) and der(22q) deletions. Hagemeijer *et al*. [7] and Nacheva *et al.* [21] postulated a two-step rearrangement for the localisation of *BCR*/*ABL* gene on chromosome 9. A *BCR*/*ABL* translocation occurs initially, followed by a translocation between the derivatives 9 and 22. This results in the masking of the exchange, with the *BCR*/*ABL* transposition to chromosome 9. Alternatively, it may involve an insertion of the *BCR* gene from chromosome 22 into the *ABL* gene on chromosome 9 [22]. Indeed, Sinclair *et al*. [16] found that deletions of the proximal sequences on the derivative chromosome 9q were associated with a poor prognosis on standard drug therapy. In addition, it has been shown that the deletion is not acquired during disease progression as the deletion tends to be consistent throughout the course of the disease [19]. However, no distinctive clinical features were found in the patients with typical or atypical transcripts. At the same time, the typical 1F2R1G rearrangement of *BCR*/*ABL* in AML, ALL and MDS was observed in 4 cases (**Table 4**). The atypical patterns (1F1R1G) were also found in two AML cases (50%). ALL and MDS cases did not show an atypical iFISH pattern (**Table 4**). We did not observe extensive translocations, deletions and invertion of chromosomes 9 and 22 that can arise during follow up. However, in an other study, the most common atypical iFISH pattern among Ph-positive patients was seen to be 1F1R1G rearrangement [23]. Lawce *et al.* [24], for this subgroup of patients, detection of residual disease is difficult since the pattern is indistinguishable from juxtaposition artifacts as a result of random overlapping between chromosomes 9 and 22. Primo *et al.* [23] and Lim *et al.* [13] demonstrated the presence of such a pattern in about 12.5%, 9% and 10.4% respectively. For example, a 1F1R1G pattern at diagnosis may change to a 2F1R2G pattern as a result of a gain of a Ph chromosome, and this may inadvertently be misinterpreted as atypical iFISH abnormal pattern while in fact it has the clinical implication of secondary clonal changes. Molecular studies have shown that the poor prognosis is related to loss of *BCR/ABL* expression, increased *BCR*/*ABL* transcripts, or genetic instability [25]. It was shown recently that there were no difference in the survival rates between patients with and without deletions when imatinib treatment was given [26]. Nevertheless, the time to disease progression was significantly shorter for patients on imatinib treatment regardless of chronic or advanced phases. In our study, none of Ph-positive patients showed a gain of a Ph chromosome. This state is not usually associated with disease progression or secondary genetic changes, and is one of the major pathways of clonal evolution seen during blast crisis. Primo *et al.* [23] showed that the presence of additional Ph chromosomes was one of the most common underlying genetic abnormalities when using FISH to identify *BCR*/*ABL* rearrangements in CML and ALL. These gains were more frequently observed in ALL than in CML.

The *AML*1 gene has recently attracted a lot of interest in terms of its role in leukemogenesis. The translocation between chromosomes 8 and 21 is the most frequent abnormality seen in approximately 46% of patients with *AML* and an aneuploid karyotype. Amplification of *AML*1 has been reported in *AML*, where structural rearrangements resulted in partial gains of chromosome 21. Acquired trisomy 21, a frequent finding in childhood ALL, itself produces one extra copy of the gene. Structural rearrangements involving duplication of the long arm of chromosome 21, have also been described [27-29]. Interestingly, we have seen that a total 35 patients with AML (18 cases), CML (7 cases), ALL (5 cases) and MDS (5 cases) were not associated with different numbers of iFISH patterns with the usage of *AML1/ETO* [t(8;21)] Dual Color Dual Fusion Translocation probe. However, the *PML/RARA* [t(15; 17)] and *CBFB*[inv(16)] probes gave positive results only for 12.5% (3/24) and 8.3% (1/12) of patients with AML, respectively, and for *p53* [del(17p13.1)] in one of two patients with CML (**Table 3**). It has been estimated that about 50% of all tumours have mutations in *p*53, and the *p*53 pathway may be nonfunctional for other reasons in many others. *p*53 is also known for its role in monitoring genomic stability, but the mechanisms underlying this function are not fully understood.

FISH can be a useful tool for monitoring remission status when clonal chromosome abnormalities have been identified at diagnosis. By conventional cytogenetic studies many of patients with myeloproliferative disorders, MDS or ALL are found to have an abnormal clone [eg, trisomy 8, monosomy 7, del(20q), etc.] that may help explain their clinical signs and symptoms. The addition of FISH to existing karyotyping procedures has led to a more definite assessment of the cytogenetic profiles of MDS and AML [30]. The sensitivity of FISH in the detection of numerical abnormalities in myeloid neoplasias has been the subject of several investigations. The most common numerical anomalies in myeloproliferative disorders are –Y, +8, +9, and –7. Trisomy 8 is a common cytogenetic abnormality found in the bone marrow of patients with myeloproliferative disorders, MDS or acute nonlymphocytic leukemia [31,32]. Just as, the secondary chromosomal aberrations (8.5% of all cases) are clearly nonrandom in the present study; with the most common chromosomal abnormalities being +8 (62.5% of cases

with additional changes), +21(25%), del(17)(12.5%), and monosomy 9(12.5%) by interphase FISH. We suggest that all these aberrations, occurring in 75% of AML cases, 12.5% MDS and 12.5% of ALL with secondary changes, should be denoted major route abnormalities. Among the approximately 1600 CML cases with standard Ph and secondary changes published to date [33], +8, +Ph, i(17q) and +19 have been described in 35, 31, 21, and 14% of the cases, respectively. The incidence of trisomy 8 in 140 Chinese patients with CLL were found in only two patients (1.4%) [34]. Our study demonstrates that trisomy 8 was not found in CLL, and its role in prognosis of CLL remains unknown. Using conventional cytogenetic methods in which 20 to 30 metaphase cells are typically examined, it is possible to find 2 or more metaphases with trisomy 8 in many of these patients. According to the ISCN definition, 6 these specimens would have an abnormal clone. In our study, +8, –9 and +21 are clonal by ISCN criteria and may not reflect either artifact or the potential emergence of an abnormal clone. All other additional chromosomal abnormalities occur in less than 10% of the CML cases, the most frequent being –Y, +21, +17, –7, and –17 [35]. Anastasi *et al.* [36] demonstrated that of three patients with MDS associated with trisomy 8. Structural rearrangements involving duplication of 21q, dup(21q) have also been described [27-29]. Combining these myeloid disorder groups and our findings, the most common additional chromosomal changes are +8, +Ph, i(17q), del(17), +19, –Y, +21, +17, –9 and –7. These abnormalities were proposed to follow the major route of clonal evolution, whereas other changes evolving more rarely were suggested to follow the minor route [37]. In view of our present knowledge, it may be reasonable to expand the major evolutionary route to include all these aberrations, using 5% as a reasonable cut-off value. Our study shows that FISH analysis using directly labeled DNA probes specific for chromosome 8 is an excellent way to detect trisomy 8 cells in hematologic malignancies. iFISH could also be used to monitor the effects of treatment, in patients found to have trisomy 8 at the time of diagnosis, or to detect early relapse. We believe this study predicts that such analyses could be extended to other chromosomal trisomies and monosomies; although the latter will require even stricter attention to normal value studies.

In conclusion, our results confirmed that iFISH has become an invaluable tool in defining and monitoring acquired chromosome abnormalities associated with hematologic malignancies, and is a sensitive technique for the evaluation of response to treatment in patients with CML. Despite the high incidence of typical iFISH patterns of *BCR*/*ABL* gene rearrangements, atypical patterns are also found in *BCR*/*ABL*+ CML and AML. Moreover, our study suggests that FISH analysis using directly labeled DNA probes specific for chromosome 8 is an excellent way to detect trisomy 8 cells in hematologic malignancies, or it could be used to supplement conventional cytogenetic studies in patients with normal or uncertain results.

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