

DETECTION OF CHROMOSOMAL ABNORMALITIES ON INTERFACE CELLS FROM MYELOID LEUKEMIAS BY FISH

Kürşat Özdilli Assist. Prof. PhD. MD,^{1,2} Filiz Aydın Prof. PhD. MD,² Sevgi Kalayoğlu Beşışık Prof. MD,³ Fatma Oğuz Prof. PhD. MD,² Sonay Temurhan,² Özüm Çako,² Halim İşsever Assoc. Prof. PhD.⁴

¹ Halic University, Institute of Health, Mecidiyeköy, Istanbul

² Istanbul University, Istanbul Medical School, Department of Medical Biology, Capa, Istanbul

³ Istanbul University, Istanbul Medical School, Department of Internal Medicine, Haematology, Capa, Istanbul

⁴ Istanbul University, Istanbul Medical School, Department of Public Health, Capa, Istanbul

ABSTRACT

• **Objective:** Chronic myeloid leukemia (CML) was the first cancer associated with specific cytogenetic abnormalities, and a variety of chromosomal abnormalities in acute myeloid leukemia (AML), such as numerical and structural alterations and balanced translocations, have also been described.

• **Material and Method:** In this study, blood or bone marrow samples of 99 patients diagnosed with myeloid leukemia between 2000-2006 (CML n=57, AML n=42) were evaluated retrospectively using fluorescent in situ hybridization (FISH). All AML and CML patients included in the study group were analyzed for trisomy 8, Philadelphia (Ph) chromosome were analyzed in 57 CML patients, and 34 of the AML patients were analyzed for -7/del7q and -5/del5q abnormalities.

• **Results:** Of the 99 patients diagnosed with CML and

AML, 53.5% were male and 46.5% were female, with an average age of 40±17 years. The presence of +8 was detected in 29.8% of the CML patients, and 70.2% tested positive for Ph chromosome. The Ph+ chromosome ratio was 82.1% in male patients and 58.6% in female patients ($p<0.05$; OR=0.3). The +8 abnormality was detected in 46.34% of AML patients, but there was no significant difference in the +8 frequency between males and females ($p=0.8$). The -7 abnormality was the only abnormality detected in three AML patients; +8, del7q and -5 were detected in one patient; +8 and del7q were detected in two patients; and -5 alone was detected in one patient.

• **Conclusion:** In conclusion, the high frequency of +8 in CML and AML cases highlights the necessity for routine analysis of these abnormalities.

• **Key Words:** FISH, chromosomal aberration, myeloid leukaemia Nobel Med 2010; 6(3): 84-89

FISH YÖNTEMİ İLE MİYELOİD LÖSEMİ HÜCRELERİNİN ARAYÜZEYLERİNDEKİ KROMOZOMAL ANOMALİLERİN SAPTANMASI

ÖZET

• **Amaç:** Kronik miyeloid lösemi (KML) sitogenetik anomaliler ile ilişkisi tanımlanan ilk kanserdir. Akut miyeloid lösemi olgularında (AML) ise kromozomal anomaliler sayısal ve yapısal değişiklikler ya da dengeli translokasyon şeklinde çeşitlilik göstermektedir.

• **Materyal ve Metod:** 2000-2006 tarihleri arasında myeloid tip lösemi tanısı alan 99 hastanın (KML n=57, AML n=42) kan örneklerinde FISH yöntemi ile yapılan çalışmanın analiz sonuçları retrospektif olarak değerlendirilmiştir. Çalışma grubuna alınan hastaların tümü +8 açısından AML hastalarından 34'ü ise -7/del7q ve -5/del5q varlığı açısından değerlendirilmiştir. +8 taraması için CEP8 (Vysis) probu, -7/del7 için LSI D7S522 (7q31), Spectrum Orange/CEP 7 Spectrum Green probe (Vysis), -5/del5q için LSI CSF1R (5q33-q34) SpectrumOrange/D5S23, D5S721 SpectrumGreen probe (Vysis) kullanılmıştır.

• **Bulgular:** KML ve AML tanısı olan 99 hastanın %53,5'i erkek, %46,5'si kadın olup, yaş ortalaması 40 ± 17 yıl olarak hesaplandı. KML'li hastaların %29,8'inde +8 varlığı belirlenirken, %70,2'sinde Ph + olarak saptandı. Bu oran erkek hastalarda %82,1, kadın hastalarda ise %58,6 bulundu ($p < 0,05$; OR: 0,3).

AML'li hastaların %46,34'ünde +8 saptanmış ancak cinsiyet açısından +8 sıklığı değerlendirildiğinde bir anlamlılık bulunamamıştır ($p=0,8$). Üç AML hastasında -7 tek anomali olarak görülürken, bir hastada +8, del7q ve -5, iki hastada +8'e ve del7q ve bir hastada sadece -5 saptanmıştır.

• **Sonuç:** Sonuç olarak FISH yöntemi ile KML ve AML hastalarımızla yaptığımız bu çalışma ile hastalıkla ilişkili prognostik olarak önemli anomaliler ile ilgili sonuçlarımızı değerlendirdik. Özellikle +8 sıklığının KML ve AML vakalarında oldukça yüksek olmasının rutin analizler sırasında bu anomalilerin de analizinin gerekliliğini vurguladığı düşüncesindeyiz.

• **Anahtar Kelimeler:** FISH, kromozomal anomaliler, myeloid lösemi *Nobel Med 2010; 6(3): 84-89*

INTRODUCTION

Cytogenetic analysis is important for establishing the diagnosis and determining the prognosis of many haematological diseases, including myelodysplastic syndrome.¹ Chronic myeloid leukaemia (CML) was the first cancer associated with a specific cytogenetic abnormality. The Philadelphia chromosome is formed following a reciprocal translocation between chromosome 9 and chromosome 22 [t;(9;22)(q34;q11)] resulting in a fusion between the c-abl gene on chromosome 9 and the bcr gene region on chromosome 22.²⁻⁶ The bcr-abl gene product has protein kinase activity that leads to the proliferation of factor-independent bone marrow

cells. In acute myeloid leukaemia (AML), the karyotype is an important prognostic parameter, and classification of AML cases is carried out based on the presence of cytogenetic abnormalities, which aids in determining the clinical course.⁷ Karyotypic abnormalities also provide important information for the identification of residual diseases.⁸ There are various chromosomal abnormalities present in AML cases, including numerical alterations, structural alterations and balanced translocations.⁹

Conventional cytogenetic analysis (G-banding) depends on the presence of cells undergoing division. Therefore, it fails to identify chromosomal abnormalities in cases with low mitotic indices or deficiencies in cytogenetic sample preparation.¹⁰ In fact, during the remission period following chemotherapy in acute and chronic leukaemia, reduced mitotic activity results in reduced sensitivity of this method.¹¹ In contrast, with fluorescence in situ hybridization (FISH), the nondividing interphase nucleus is studied. Furthermore, samples prepared for conventional cytogenetic analysis can be evaluated using FISH without requiring new sample preparation.

In this study, AML and CML cases were evaluated for various chromosomal abnormalities using a routine FISH method. CML patient samples were analysed for the presence of the Ph chromosome and trisomy 8, and AML patient samples were analysed for the presence of →

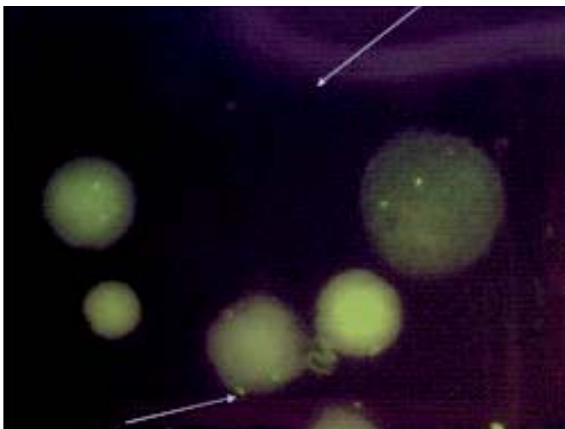


Figure 1. Ph-positive cells

Table 1: Chronic myeloid leukaemia patients				
Patient no	Age	Sex	Ph chromosome (%)	Trisomy 8 (%)
1	46	M	81	5
2	34	F	41	N
3	49	F	N	14
4	56	M	8	N
5	40	M	90	11
6	42	F	N	N
7	45	M	N	N
8	49	M	42	N
9	61	M	40	N
10	16	F	6	N
11	53	F	32	N
12	32	M	22	10
13	64	F	N	7
14	52	M	81	6
15	51	F	74	N
16	37	M	80	N
17	45	M	60	N
18	73	M	10	N
19	43	M	92	N
20	55	M	N	N
21	19	F	5	N
22	14	M	N	N
23	43	M	8	N
24	72	M	45	N
25	65	M	10	N
26	37	F	N	N
27	57	F	24	N
28	45	F	N	N
29	53	F	5	N
30	25	F	10	N
31	44	F	18	N
32	33	F	24	N
33	30	F	14	N
34	22	M	8	7
35	52	M	16	N
36	59	F	N	N
37	58	F	N	N
38	41	M	N	7
39	37	F	N	N
40	53	M	14	6
41	45	F	7	8
42	46	M	9	6
43	29	F	11	N
44	30	F	6	N
45	72	M	10	N
46	23	F	9	N
47	21	M	16	N
48	69	F	10	N
49	62	F	N	6
50	54	F	N	N
51	46	F	N	5
52	49	M	N	10
53	43	F	N	5
54	47	M	15	5
55	53	F	8	6
56	51	M	10	N
57	57	M	93	N

del5, monosomy 5, del7, monosomy 7 and trisomy 8, with the aim of determining the distribution of chromosomal abnormalities in our patient population. Our aim is to assess detailed examination and analysis of the prevalence of these abnormalities in our patient population with the implementation of FISH method

MATERIAL and METHOD

Samples from 99 patients (CML n=57, AML n=42), originally seen between 2000 and 2006, were analyzed using FISH at the FISH Laboratory of Istanbul University Istanbul School of Medicine Department of Medical Biology.

FISH was performed on bone marrow or peripheral blood samples. The FISH procedure is performed according to the manufacturer's protocols. In brief target DNA was affixed to glass slides and probe DNA were denatured in 70% formamide (pH 7.0) at 73°C for 5 minutes, then slides were passed through cold ethanol series and were allowed to dry. Probe was placed on the target DNA and the slides were incubated overnight at 37°C. After post-hybridization, they were washed for 2 minutes in 0.5xSSC at 72°C and twice for 3 minutes in phosphate - buffered detergent at room temperature. Finally, slides were counterstained with DAPI. The slides were viewed by a fluorescence microscope (Nikon E800) with suitable filter set.

Indefinite signals and nuclei with poor morphological displays were excluded from the evaluation. Signals from 500 nuclei were evaluated for each slide. The t;(9;22)(q34;q11) translocation in CML patients was investigated using a dual-colour BCR/ABL (Vysis) probe. The LSI D7S522 (7q31) Spectrum Orange/CEP 7 SpectrumGreen probe (Vysis) was used to investigate monosomy 7 and del7, and the LSI CSF1R (5q33-q34) Spectrum Orange/D5S23, D5S721 Spectrum Green probe (Vysis) was used to investigate monosomy 5 and del5q. Trisomy 8 scanning was carried out using the CEP8 (Vysis) probe on samples from CML and AML patients undergoing treatment.

RESULTS

Among the 99 patients diagnosed with CML and AML, 53.5% (53) were male and 46.5% (46) were female. The average patient age was 40±17 years. Forty-two (42.4%) patients included in the study had AML, and 57 (57.6%) had CML.

Chronic myeloid leukaemia cases: The female to male ratio of the 57 CML patients (F/M) was 29:28, and the average age was 45.60±14.27 years. Trisomy 8 was detected in 29.8% (n=17) of the CML patients, and →

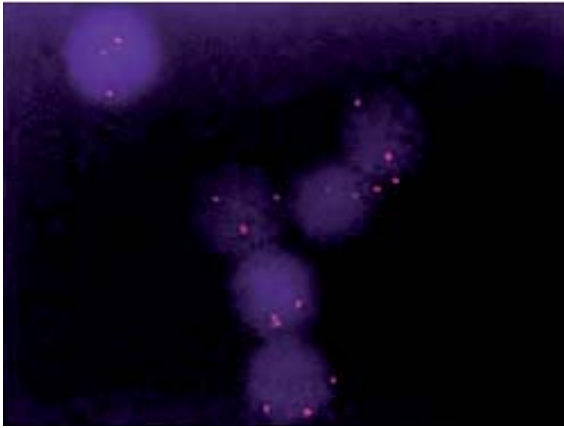


Figure 2. Trisomy 8-positive cells

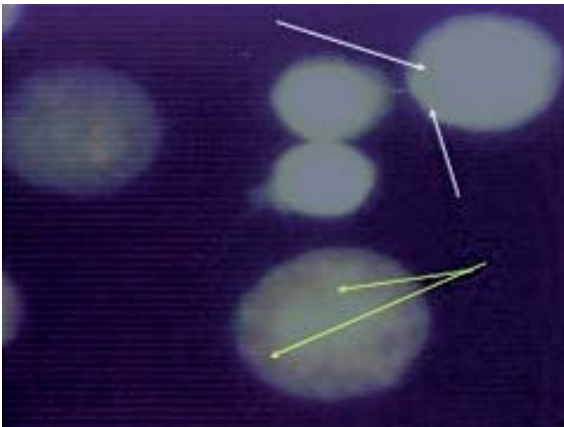


Figure 3. Normal cells (2 red, 2 green signals) and cells with deletion (1 green, 1 red signal) were detected

70.2% (n=40) were positive for Ph chromosome (Figure 1). The Ph+ chromosome ratio was 82.1% (n=23) for male patients and 58.6% (n=17) for female patients. (p=0.05; OR=0.3). (Table 1, Figure 2).

Acute myeloid leukaemia cases: The female to male ratio of the 42 patients (F/M) was 17:25, and the average age was 32.48±18 years. The study was carried out using samples collected during clinical diagnosis. Trisomy 8 was detected in 46.34% (n=19) of the patients; there was no significant difference in the trisomy 8 frequency between males and females (p=0.8). Analyses of -7/del 7q and -5/del5q were carried out for 34 patients. Monosomy 7 was the only abnormality detected in three patients (patients 19, 20 and 35). In one patient (patient 9), +8, del7q and -5 were all detected; in two patients (patients 13 and 41), trisomy 8 and del 7q were both detected; and in one patient, only monosomy 5 was detected (Table 2, Figure 3).

DISCUSSION

FISH and Polymerase Chain Reaction (PCR) are efficient methods for detecting genetic differences in the karyotype of patients with hematologic disorders.¹²⁻¹⁵ The sensitivity and efficiency of inter-phase FISH and G-banding

Table 2: Acute myeloid leukaemia (AML) patients

Patient no	Diagnosis	Age	Sex	Trisomy 8 (%)	-7	del 7q (%)	-5 (%)	del 5q	BMT
1	AML-M2	41	M		N	N	N	N	
2	AML	36	M	5	N	N	N	N	BMT
3	AML	32	M		N	N	N	N	
4	AML-M4	32	F		N	N	N	N	BMT
5	AML-M3	60	M		N	N	N	N	
6	AML	10	M		N	N			
7	MDS	10	F	6	N	N	N	N	BMT
8	AML-M4	29	F		N	N			BMT
9	AML-M4	7	F	6	N	16	5	N	
10	AML-M2	38	M	7	N	N	N	N	BMT
11	AML-M3	34	M		N	N	N	N	
12	AML-M3	25	F		N	N	N	N	
13	AML	33	M	8	N	8	N	N	BMT
14	AML-M4	57	F	8			N	N	
15	AML	36	M	5	N	N	6	N	BMT
16	AML-M3	66	M	5	N	N	N	N	
17	AML	12	F	5	N	N	N	N	
18	AML	13	M	11	N	N	N	N	
19	AML-M4	16	M		6	N	N	N	
20	AML-M3	31	F		9	N	N	N	
21	AML-M1-M2	40	M		N	N	N	N	
22	AML	29	M		N	N	N	N	
23	AML-M3	31	F	6					
24	AML	51	F						BMT
25	AML-M4	50	M	6	N	N	N	N	
26	AML-M5	40	M	88	N	N	N	N	
27	AML	11	F	5	N	N	N	N	
28	AML-M3	19	M	8	N	N	N	N	
29	AML	74	M		N	N	N	N	
30	AML	20	M						
31	AML-M3	65	M	6	N	N	N	N	
32	AML-M4	11	M	87					
33	AML-M3	15	F						
34	AML	11	M		N	N	N	N	
35	AML	13	M		6	N	N	N	BMT
36	AML	25	F		N	N	N	N	
37	AML	16	F	89	N	N	N	N	
38	AML	32	F						
39	AML	55	M						
40	AML-M3	44	F		N	N	N	N	
41	AML-M3	31	F	7	N	11	N	N	
42	AML-M3	63	M		N	N	N	N	

M: Male, F: Female, N: Number, BMT: Bone marrow transplantation

analyses was compared in several studies.¹⁰⁻¹² Several investigators reported that FISH method has many advantages compared to G banding as it provides precise detection of multiple cells in a shorter time and does not require the presence of dividing cells.

FISH is also helpful for determining the percentile ratio of minimal residual cells. The term “relapse of disease” is used when the myeloblast ratio is more than 20% after chemotherapy.¹⁶ →

Trisomy 8 is frequently detected with a secondary chromosome abnormality in myeloid leukemias. In a study of Schoch et al. with 713 AML patients, trisomy 8 was the most frequent numerical chromosomal aberration occurring either as the sole abnormality or together with other chromosome aberrations, frequently detected with a secondary chromosome abnormality.¹⁷ The chromosome abnormality frequencies detected in a large group of AML patients in a study by Grimwade and colleagues were as follows: trisomy 8 (9%), monosomy 7 (3%), del 7q (2%), del 5q (2%) and, monosomy 5 (2%).¹⁸

In the present study, the interphase FISH method was used to retrospectively investigate 42 AML patients for trisomy 8, the most common abnormality detected. The +8 abnormality was detected in 46.34% of AML patients, but there was no significant difference in the trisomy 8 frequency between males and females (p=0.8).

We performed FISH analyses for -7/del7q, -5/del5q only in 34 of 42 AML patients due to insufficient sample. The Monosomy 7 was the only abnormality detected in three AML patients; trisomy 8, del7q and -5 were detected in one patient; +8 and del7q were detected in two patients; and -5 alone was detected in one patient. In a study of Grimwade et al., monosomy 7 was detected in 3%, monosomy 5 was detected in 2% of the patient group. Compared to previous study, in our study group it was found out that the frequency of monosomy 7 was comparatively higher with 8.8% whereas that of monosomy 5 was close to the earlier study with 2.4%. Trisomy 8 is a common clonal formation in CML progression. The association between various stages of t(9;22) leukaemias and trisomy 8 has not been strongly established.¹⁹ Johansson et al. reported that t(9;22) is often detected as the only abnormality in the chronic disease phase, whereas additional genetic alterations occur in 60-80% of patients in blastic crisis. In cases with additional alterations, trisomy 8, +Ph, i(17q), +19,

-Y (in males), +21, +17 and -7 were detected as secondary abnormalities, with frequencies of 34%, 30%, 20%, 13%, 8%, 7%, 5%, and 5%, respectively.²⁰

Jacob et al. showed that the additional chromosomal alterations identified during blastic crisis were extra Ph chromosomes, resulting in trisomy 8 and trisomy 19.²¹ In a study that investigated the incidence of trisomy 8 in CML patients, the frequency was 64% in myeloid blastic crisis and 39% in the accelerated phase. The lowest frequency (13%) was found in samples taken from three patients who were morphologically in the chronic phase.²²

Our CML patient samples were analysed for the presence of the Ph chromosome and trisomy 8. Trisomy 8 was detected in 29.8% (n=17) of the CML patients, and 70.2% (n=40) were positive for Ph chromosome. In 10 of 57 patients (patients 1, 5, 12, 14, 34, 40, 41, 42, 54 and 55), trisomy 8 (17.5%) was detected along with t(9;22). This frequency was lower than the frequency of trisomy 8 reported as a secondary abnormality in blastic crisis by Johansson et al. This might be explained by the fact that we evaluated patients for the presence of trisomy 8 at different levels of disease progression.

CONCLUSION

In conclusion, we analysed CML and AML patient samples using FISH and evaluated the presence of the most common and prognostically important abnormalities. We believe that the high frequency of +8 in CML and AML cases underlines the need for analysing this abnormality during routine analysis. We believe that FISH is an effective and sensitive methodology in detecting this abnormality and it is quiet applicable in following minimal residual diseases, in this sense it is safe to claim that FISH should be benefited for routine analysis of relevant diseases.

C	CORRESPONDING AUTHOR: Kürşat Özdiilli, Assist. Prof. PhD. MD Halic University, Institute of Health, Mecidiyekoy, Istanbul ozdililk@yahoo.com
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