Original article

Atypical patterns of BCR/ABL gene rearrangements by interphase fluorescence in situ hybridization (FISH) in patients with chronic myeloid leukemia

Prezența unor aspecte atipice ale genei BCR/ABL puse în evidență prin FISH la pacienții cu leucemie mieloidă cronică

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Abstract

Fluorescent in situ hybridization (FISH) technique is increasingly used for the identification of BCR/ABL gene rearrangements in chronic myeloid leukemia (CML).Patients and Methods: Both typical and atypical pattern of BCR/ABL gene rearrangements was determined in 83 patients with CML referred to Fundeni Clinical Institute using dual fusion fluorescence in situ hybridization (DF-FISH) probes and conventional cytogenetics.Results: in the majority of cases - 82 out of 83- CML patients exhibited a translocation between chromosome 9 and 22, resulting in a Philadelphia chromosome and one patient, negative for the Ph chromosome, presented cryptic BCR/ABL rearrangement. All samples were analyzed both by FISH and conventional cytogenetics. In 71 out of 83 cases the typical interphase FISH signal patterns of BCR/ABL gene rearrangements was identified – one red signal, one green signal and two fusion signals (1R1G2F). The rest of 11 patients presented an atypical interphase FISH pattern. Atypical patterns included: loss of terminal region of derivative chromosome 9 (five patients), loss of derivative chromosome 9 (del(9q)der) (three patients), isodicentric 22 derivative chromosome fused at terminal region of q arm (one patient), isoderivative chromosomes 22 (ider(22)t(9;22)(q34;q11)) (one patient) and supernumerary Philadelphia (one patient).Conclusion: FISH technique exhibited an advantage of being simple and still allowing the identification of cryptic BCR-ABL insertion or variant Ph. Another advantage of this technique is that it allows examination of interphase nuclei in cases were no metaphases could be obtained and atypical patterns may have clinical prognostic implication.

Keywords: *peripheral blood-FISH technique, BCR/ABL gene fusion, minimal residual disease*

Rezumat

Tehnica FISH este din ce in ce mai frecvent utilizată în identificarea rearanjărilor genei BCR/ABL la pacienții cu leucemie mieloidă cronică. Pacienţi şi Metode: Determinarea aspecte tipice şi atipice ale genei

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BCR/ABL a fost realizata la un numar de 83 de pacienți internați in Institutul Clinic Fundeni utilizând atât prin tehnica FISH (sonde FISH cu 2 semnale de fuziune) cât şi prin citogenetica conventională. Rezultate: Au fost investigați prin examen citogenetic si tehnica FISH 83 de pacienți. Cu excepția unui singur pacient diagnosticat Philadelphia-negativ prin citogenetica conventională (cu rearanjare criptică a genei BCR/ABL pusa in evidenta prin tehnica FISH), restul de 82 au prezentat translocația (9;22) (cromozomul Philadelphia). Dintre aceştia, 71 de pacienți au avut un aspect tipic al genei de fuziune BCR/ABL (exprimat prin prezenta unui semnal rosu (cromozomul 9), unui semnal verde (cromozomul 22) si a doua semnale galbene rezultat din fuziunea celor doua gene de la nivelul cromozomilor 9 si 22) – 1R1V2F iar 11 pacienți au prezentat un aspect atipic. Aspectele atipice puse în evidență au prezentat: pierderea regiunii terminale a cromozomului 9 derivativ (del(9q)der) (cinci pacienti), pierderea cromozomului 9 derivativ (trei pacienti), cromozom 22 derivativ isodicentric (un pacient), cromozom 22 isoderivativ (ider(22)t(9;22)(q34;q11)) (un pacient) si Ph supranumerar (un pacient). Concluzii: Tehnica FISH are avantajul că este o tehnică simplă şi că permite punerea în evidență a rearanjărilor criptice ale genei BCR/ABL. Aspectele atipice puse în evidență prin această tehnică sunt utile în clinică pentru prognostic. Examinarea nucleilor interfazici în cazurile la care nu sunt obținute metafaze reprezintă un avantaj în plus al tehnicii FISH.

Cuvinte cheie: *boală minimaă reyiduală, fuyiunea genelor BCR/ABL, tehnica FISH din sâange ăperferic*

Introduction

Cytogenetic analysis is a standard method for chronic myeloid leukemia (CML) diagnosis. This method is especially useful as it not only identifies the landmark sign of CML – Philadelphia (Ph) chromosome, but also allows identification of additional karyotype abnormalities. In 95% of CML cases at diagnosis, standard cytogenetics identifies a Ph chromosome which bears BCR-ABL1 fusion gene (1-4). In the remaining 5% of the cases no Ph chromosome could be detected. In these cases CML patients carry a cryptic translocation detectable by molecular techniques such as FISH or PCR (Polymerase Chain Reaction) (5).

FISH analysis is important as an ancillary technique for CML evaluation, in combination with standard cytogenetics, as it identifies cryptic translocations and also displays a higher level of sensitivity in case of monitoring of minimal residual disease (MRD). Also, FISH technique is useful in cases where no metaphases can be obtained, as it allows examination of interphase nuclei.

Initially, single fusion FISH probes were described which allowed identification of fusion event between BCR and ABL1 genes. Fusion is identified as a yellow signal which results from partial overlap of green and red fluorescence. Unfortunately, these probes displayed a high level of false positive results due to overlap of probes in nuclei. False positive rate was around 15% which was considered unacceptable especially for MRD evaluation (6). Thus technical artifacts limited the potential of single fusion FISH probes to detect and quantify minimal residual disease accurately.

Dual fusion FISH probes were introduced to overcome technical limitations of single fusion ones. These probes are made so that each probe (on 9q and 22q) span the breakpoint region, so that translocation (9;22) generates two fusion gene signals: one on the Ph chromosome and one on the 9th derivative chromosome. This improvement of FISH probes allows identification of Ph chromosomes with high level of confidence generally reducing level of false positives to almost nothing (7).

Additional chromosome abnormalities in CML can cause disruption of a typical CML FISH pattern: one red, one green and two fusion signals (8, 9). In this article we describe abnormal FISH patterns identified in CML patients which were confirmed and explained by standard cytogenetics as additional chromosome abnormalities.

Patients and methods

A total of 83 patients referred to Fundeni Clinical Institute were included in the study. A

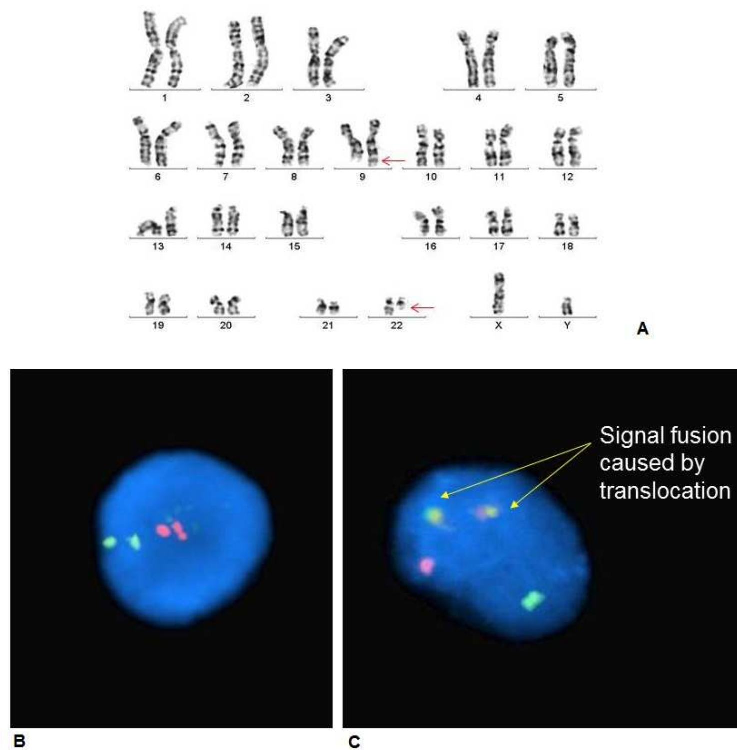

Figure 1. A. Karyotype 46,XY,t(9;22); B. A normal cell with two clearly separated Green (BCR probe) and Red (ABL probe) signals; C. Ph-positive cell with one Red signals, one Green signal, and two Yellow signal

number of 21 samples derived from patients at the onset of the disease and 62 samples derived from patients during the course of therapy. In all cases, heparin-anticoagulation fresh bone marrow (BM) aspirate samples were obtained and used for the cytogenetic examination and FISH analysis.

Conventional Karyotyping

Bone marrow samples were cultured using overnight and synchronized culture and processed by conventional cytogenetic procedures with GTG banding (G-bands by trypsin and Giemsa) (10). In each case, at least 20 metaphases were analyzed and the karyotypes were described according to ISCN 2009 (11).

FISH Analysis

FISH analysis was performed on metaphases and interphase cells using a dual-color BCR/ABL probe, provided by Cytocell, Cambridge, UK. After slide preparation, cells were aged by incubation overnight at 37°C. FISH analysis of cultured BM samples was performed using slides obtained by usual protocol for cytogenetic examination. Slides were dehydrated in an alcohol series. Co-denaturation was carried out for 2 minutes at 75°C, followed by overnight hybridization at 37°C. After overnight hybridization slides were washed in 0,4X SSC at 73°C for 2 minutes and rinsed in 2 X SSC. Evaluation of the FISH signals was performed using a fluorescence microscope (AxioImager, Zeiss, Germany). For each case, a minimum of 200 interphase nuclei was evaluated.

Results

Evaluation of samples using conventional cytogenetics from 83 CML patients showed that in 82 cases a translocation between chromosome 9 and 22, resulting in a Philadelphia chromosome, was identified. Only one patient was negative for the Ph chromosome but positive for

Table 1. Atypical iFISH patterns in Ph positive patients

FISH pattern with DF-FISH probes	Chromosome localisation of signals			Interpretation
	Fusion	Red	Green	
1F1R1G	1F(Ph)	1R(9)	1G(22)	t(9;22) loss of residual proximal 9q
3R2G/1F 1R1G	1F(Ph)	R(9)	G(22)	Trisomy 9; loss of residual proximal 9q
3F1R1G	3F(Ph,Ph,der9)	R(9)	G(22)	t(9;22);+Ph
5F1R1G	5F(der9;2ider22)	R(9)	G(22)	der(9); 2ider(22)t(9;22)(q34;q11)
3F1R1G	3F(der9;idic(22))	R(9)	G(22)	der(9); idic(22)t(9;22)(q34;q11)

ider: iso derivative; idic: iso dicentric; Ph: Philadelphia; F: fusion; R: red; G: green

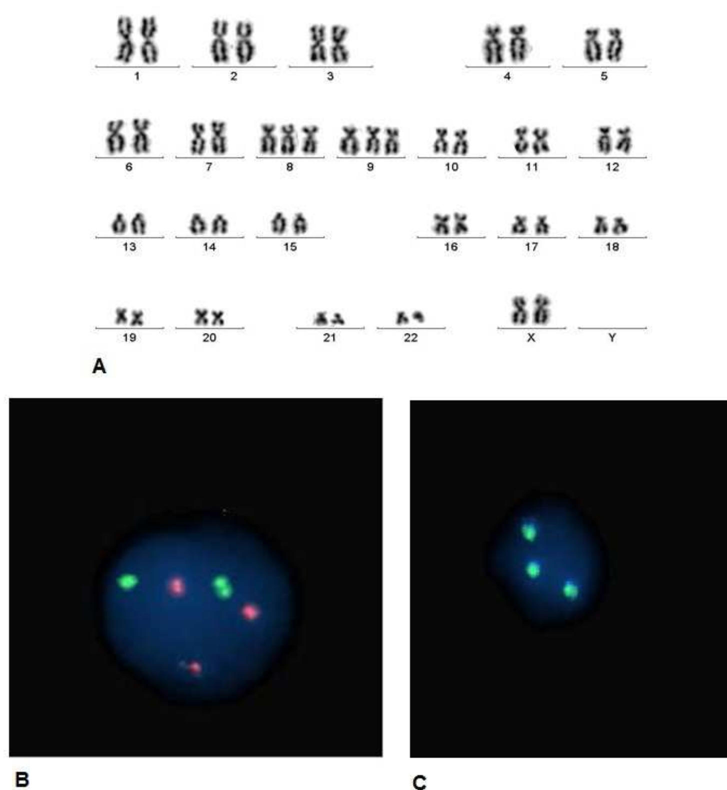

Figure 2. A. Karyotype 48,XX,+8,+9; B. A cell with two clearly separated Green (BCR probe) and three Red (ABL probe) signals; C. Cell with nuc ish (CEP8 x 3)

BCR/ABL fusion gene identified by interphase FISH and confirmed by RT-PCR (Reverse transcription polymerase chain reaction).

The majority of patients who exhibited Ph chromosome (71 out of 83) displayed the typical interphase FISH signal patterns of BCR/ABL gene rearrangements - 1R1G2F. Normal FISH pattern was confirmed by standard cytogenetics (except for the cryptic insertion) (*Figure 1*). In the rest of 11 patients atypical interphase FISH patterns were observed. *Table 1* shows the types of atypical interphase FISH patterns in Ph positive patients.

The first patient exhibited two abnormal interphase FISH patterns - 3R2G and

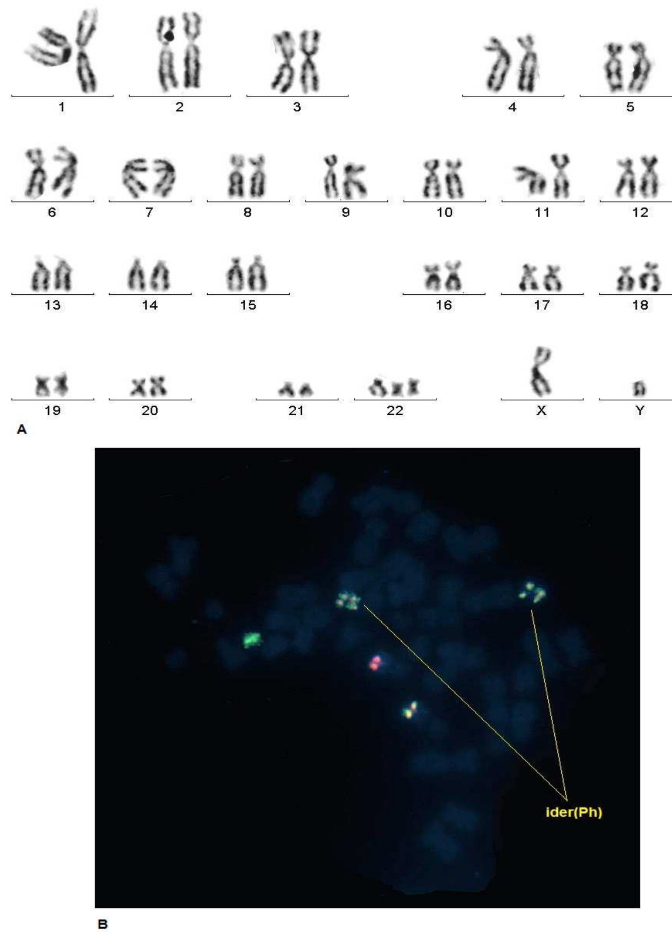

Figure 3. A. Karyotype 47,XY,der(9),2ider(22)t(9;22)(q34;q11) B. A cell with one Green signal (BCR probe, ch 22) and one Red (ABL probe,ch 9) signals and 5 Fusion signals.

1R1G1F. Cytogenetic evaluation revealed two cell lines – one with trisomy 9, trisomy 8 and without Ph chromosome and the other with Ph chromosome and absence of derivative chromosome 9. Trisomy 8 was also confirmed by FISH analysis (*Figure 2*).

The second patient exhibited 1R1G5F FISH pattern which corresponded to 47, XY, der(9),2 ider(22)t(9;22)(q34;q11) karyotype; 4 out of 5 fusion signals are due to two iso derivative 22 chromosome (the fifth fusion is due to derivative chromosome 9) (*Figure 3*).

The third patient exhibited 1R1G3F FISH pattern. This pattern corresponded to 46,XX, der(9), idic(22)t(9;22)(q43;q11); 2 out of 3 fusion signals are due to iso dicentric derivative 22 chromosome fused at terminal region of q arm. The third fusion signal is due to derivative chromosome 9 (*Figure 4*).

The fourth patient exhibited 1R1G3F FISH pattern which was explained by an additional Ph chromosome – 47,XY, t(9;22), +Ph (*Figure 5*).

In five patients 1F1R1G FISH (*Figure 6*) pattern was observed which was due to the loss of terminal region of derivative chromosome 9 – 46,XY/XX, t(9;22), del(9q?).

In another two patients 1F1R1G FISH pattern was identified, this pattern was ex-

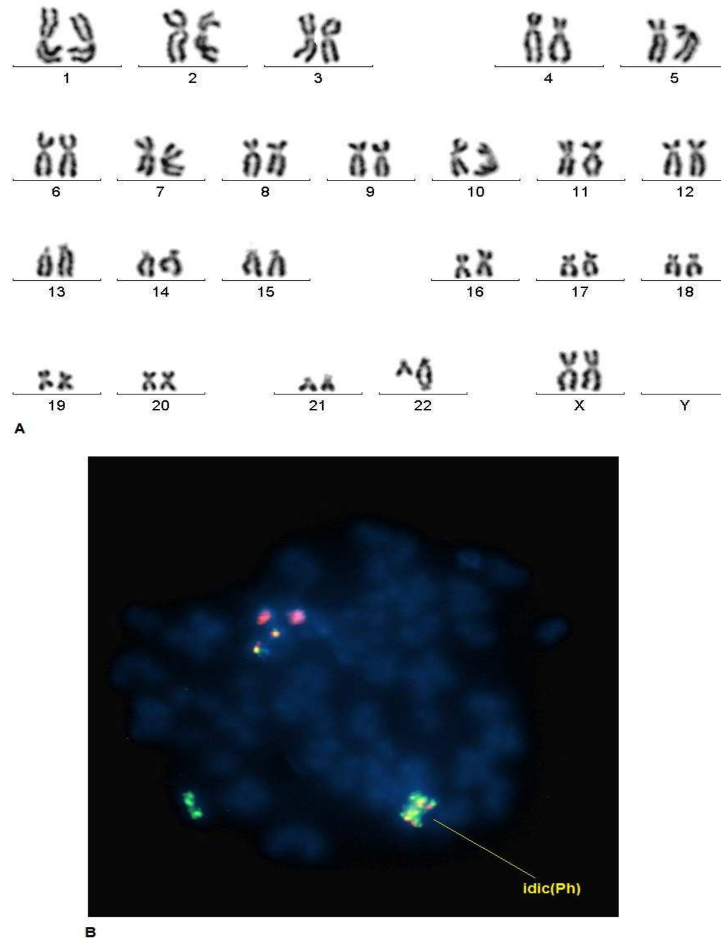

Figure 4. A. Karyotype 46,XX,der(9),idic(22)t(9;22)(q43;q11); B. A cell with one Green signal (BCR probe, ch 22) and one Red (ABL probe,ch 9) signals and 3 Fusion signals.

plained by the loss of derivative chromosome 9 - 45,XY/XX, t(9;22), -der(9) (*Figure 6*).

Discussions

The present study shows that in the most of the cases expected FISH pattern (1R1G2F) corresponded to a typical Ph chromosome as confirmed by conventional cytogenetics. To this pattern one exception, namely a cryptic BCR-ABL insertion, was noticed. Thus, the utility of double fusion FISH for CML diagnostic and monitoring was shown to be important (12). On the other hand FISH technique exhibited an advantage of being simple and rapid and allowed for the identification of cryptic BCR-ABL insertion, which is not detectable by cytogenetics. Another advantage of this technique is that it allows examination of interphase nuclei in cases were no metaphases could be obtained.

In a small fraction of patients (11 out of 83) dual fusion FISH probes generated atypical patterns as also described in the literature (8,12-14). These atypical signals were confirmed and explained by cytogenetics. In these cases additional cytogenetic changes present as aberrant pat-

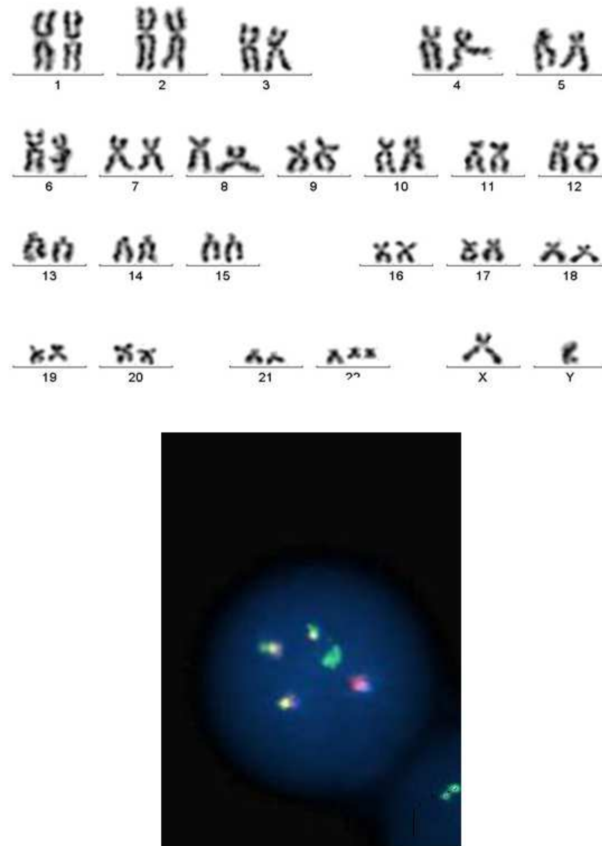

Figure 5. A. Karyotype 47,XY,t(9;22),+Ph; B. A cell with one Green signal (BCR probe, ch 22) and one Red (ABL probe,ch 9) signals and 3 Fusion signals.

terns could potentially be misdiagnosed with interphase FISH alone or could be hard to interpret with FISH on metaphases. Metaphase analyses showed that in cases with atypical interphase FISH patterns presence of an additional Ph and deletions of 9q sequences proximal to the breakpoint or of the whole derivative chromosome 9, were the two most frequent underlying genetic aberrations (12,14). Deletions of the proximal region of 9q chromosome do not represent a marker for disease progression as they tend to be consistent throughout the course of the disease (15,16). On the other hand Quintas-Cardama et al. (17) suggests that patients treated with Imatinib-mesylate can overcome negative prognostic impact of deletions of 9q chromosome region.

Gain of an additional Ph chromosome usually translated in the appearance of an additional fusion signal. Literature suggests that additional Ph chromosomes are one of the most common additional genetic abnormalities in CML (14). However, additional fusion signal could be interpreted as a gain of additional derivative chromosome 9, as fusion signal is present on both derivative chromosomes. Also an expected pattern of two fusion signals can be attributed to two Ph chromosomes coupled with the loss of derivative chromosome 9 or distal part of 9q (which is not a rare event) (12,13). Usually acquisition of an additional Ph chromosome is associated with blast crisis. Indeed, our patients was in blast crisis when FISH detected supernumerary Ph, idic(Ph) and ider(Ph).

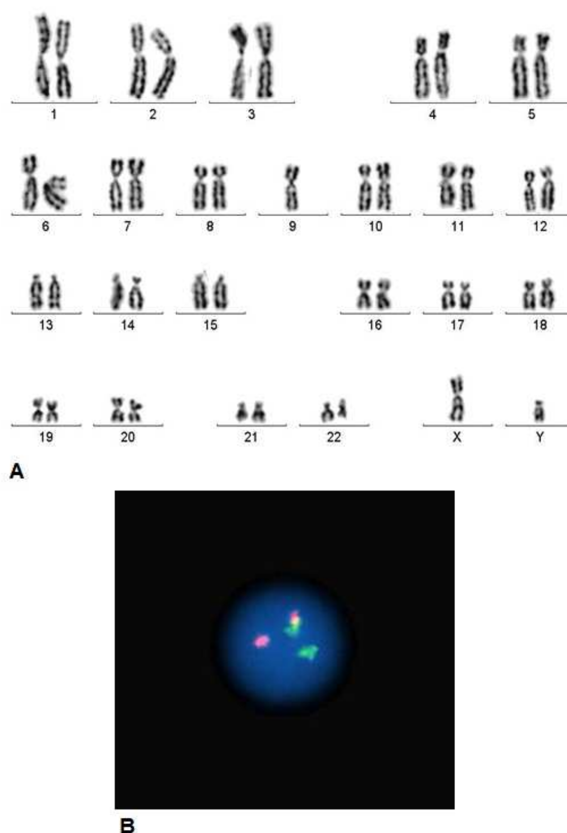

Figure 6. A. Karyotype 45,XY,t(9,22), -9; B. A cell with one Green signal (BCR probe, ch 22) and one Red (ABL probe,ch 9) signals and one Fusion signal.

In our group of patients, gain of an additional Ph chromosome translated in expected pattern of free fusion signals. In cases where an idic(22)t(9;22)(q34;q11) or two ider(22)t(9;22)(q34;q11) were present interphase FISH pattern was totally misleading and could be explained only by a combination of cytogenetics and metaphase FISH.

Conclusions

Our results indicate that interphase FISH can correctly diagnose most of Ph+ CML cases. In some rare cases exact interpretation of observed pattern requires the application of the probes on metaphases complemented with conventional cytogenetics. Acquisition of additional genetic abnormalities detected as atypical FISH pattern may have implications on clinical diagnosis and prognosis of CML patients.

Abbreviations

BM- Bone Marrow
CML- Chronic Myeloid Leukemia
DF-FISH- double fusion - Fluorescence In Situ Hybridization
FISH- Fluorescence In Situ Hybridization
GTG- G-bands by trypsin and Giemsa
ISCN- International System for Human Cytogenetic Nomenclature
Ider- iso derivative
Idic- iso dicentric
MRD- Minimal Residual Disease
PCR- Polymerase Chain Reaction
RT-PCR - Reverse Transcription Polymerase Chain

Reaction
Ph chromosome – Philadelphia chromosome
R- red; G- green; F-fusion (V-verde)

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