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# Impact of Fluorescence in Situ Hybridization in the Detection of Cryptic Fusion Transcript PML/RARA and A Complex t(5;15;17) in a Case of Acute Promyelocytic Leukemia

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## ABSTRACT

Genetic aspects of a 28 year-old female patient with typical morphological and clinical features of acute promyelocytic leukemia is presented. Pml/rara fusion transcript and a complex translocation involving chromosomes 5, 15 and 17 were detected by fluorescence in situ hybridization (FISH) technique which was applied as in adjunct to conventional cytogenetics. The patient deceased soon in spite of the immediate ATRA and cytostatic therapy.

Key Words: FISH, PML/RARA, Acute promyelocytic leukemia.

Turk J Haematol 2000;17(4):207-212.

## INTRODUCTION

Acute promyelocytic leukemia (APL) is a rare distinct subtype of myeloid leukemia characterized by invasion of bone marrow by hypergranular leukemia cells, by specific translocations almost always involving chromosome 17 and by a high sensitivity of the promyelocytic blasts to retinoic acid (RA). It has been shown to be the first model of differentiation therapy<sup>[5,6]</sup>. The disease is mainly as-

sociated with t(15;17)(q22;q12-21). The diagnosis of APL and the detection of residual disease are based on the presence of this translocation<sup>[1,3,4,5,6,7,15]</sup>. Rarely alternative balanced translocations have been described in subtypes of APL, and as more cases are being evaluated, complex, variant translocations are increasingly recognized<sup>[17,19,20,22, 24]</sup>.

Here we report a 28 year-old female patient

with typical morphological and clinical features of acute promyelocytic leukemia in whom a complex translocation involving chromosomes 5, 15 and 17 was detected by conventional cytogenetics and fluorescence in situ hybridization (FISH). Conventional cytogenetics and fluorescence in situ hybridization (FISH). Conventional cytogenetics detected a derivative 5 and a derivative 17 chromosome but failed to detect chromosome 15 involvement. Application of FISH with region specific probes and paint probes, showed that the karyotype involved a complex rearrangement of chromosomes 5, 15 and 17 including PML/RARA fusion transcript.

#### CASE REPORT

A 28 year-old female presented with massive and prolonged hemorrhagia during her menses. A complete blood count showed: hemoglobin, 70 g/L; white blood cell count,  $13.2 \times 10^9/L$ , and platelet count  $23.5 \times 10^9/L$ . Peripheral blood smear yielded more than 80% atypical promyelocytes with numerous azurophilic granules and Auer rods. Bone marrow was markedly hypercellular and also showed hypergranular atypical promyelocytes with Auer bodies which accounted for more than 90% of all nucleated cells. Coagulation tests showed a prolonged bleeding time, a normal prothrombin time: 12 seconds (control 12.3 seconds) and a borderline activated partial thromboplastin time: 35. seconds (control 34.8 seconds). Fibrin degradation products were negative in serum and urine. Massive bleeding was attributed to thrombocytopenia rather than disseminated intravascular coagulation. Immunohistochemical stainings were strongly positive for myeloperoxidase and sudan black B. Immunophenotyping showed 30% CD13 positivity and was negative for surface marker HLA-DR (10.2%). A clinical diagnosis of APL (FAB-M3) was made.

The patient was treated with all-trans retinoic acid (ATRA, 45 mg/m<sup>2</sup> daily), adriablastina (50 mg/m<sup>2</sup> for 3 days) and cytosine arabinoside (200 mg/m<sup>2</sup> for 7 days). Partial remission occurred including an increase in platelet counts and a slight decrease in leucemic cells. However at day 65 after treatment her relatives reported that she was deceased at home.

## MATERIALS and METHODS

### Cytogenetic Analysis

Cytogenetic analysis of the bone marrow aspiration was performed from direct and 24 hour nonstimulated culture preparations. Chromosomes were GTG-banded and the karyotype was described according to the International System for Human Cytogenetic Nomenclature (ISCN 1995)[11].

### Fluorescence in Situ Hybridization (FISH)

FISH studies were performed on the specimens prepared both from direct and 24-hour incubation cultures. To exclude the possibility of a cryptic t(15;17) translocation or a complex t(15;17) translocation including chromosome 5, direct labeled locus specific probe, Vysis pml/rara was used (Vysis Inc., IL, USA) Furthermore commercial digoxigenin generated chromosome paint probes (Cambio, Cambridge, UK) specific for chromosomes 5, 15 and 17 were respectively used monocolor hybridization.

Slide pretreatment was based on RNase and pepsin. For direct labeled probe pml/rara, conditions of hybridization and post-hybridization washings were performed according to Vysis' own protocol. In case of patient chromosomes, ten microliters of each probe were denatured separately at 70 °C for 10 minutes, left to preanneal at 37 °C for 1 hour and then applied to 3 different slides. After 18 hours of hybridization at 37 °C slides were washed in 50 % formamide/2xSSC. The probes were visualized using mouse anti-digoxigenin (Boehringer Mannheim) antibody. FITC signal was amplified with two additional layers of (rabbit anti-mouse), tetrahydroamine-isothiocyanate (TRITC) and (goat anti-rabbit)-TRITC. This slides were counterstained with propidium iodide (1 µg/mL). Analyses were performed under a fluorescence microscope (Axioplan Zeiss) equipped with an Applied Imaging Cytovision image analysis system (version 4.4). Both metaphase and interphase cells were examined.

## RESULTS

Cytogenetic evaluation of the bone marrow yielded a clone with a rearranged short arm of chro-

mosome 5 and a large derivative chromosome 17. By conventional GTG-banding the derivative chromosome 17 appeared to be disrupted distal to 17q21, chromosome 15 seemed to be intact, and chromosome 5 showed a short arm rearrangement. As the chromosome condensation was severe it was difficult to make a clear identification of the rearrangements, however the source of the additional material on the derivative 17q was interpreted as 5p and the karyotype was suggested to present 46, XX, t (5;17)(p13;q21)? (51 metaphases) (Fig. 1). There were also 46, XX normal metaphases (13 metaphases).

FISH studies with direct labeled locus specific probe Vysis pml/rara demonstrated three signals both on the metaphases and the nuclei: a normal green coloured chromosome 17, and a normal red coloured chromosome 15 and one double-coloured (red-yellow) signal which was suggestive of the fusion transcript pml/rara: (Fig. 2). FISH with paint chromosomes 5, 15, and 17 were also performed and in each metaphase analysed, there seemed to be the triplication of signals for each chromosome respectively (three signals for 5 three for 15 and three for 17 (Figs. 3,4,5). For each paint probe, three hybridization signals indicating chromosome 5, 15 and 17 were detected in metaphases. For chromosome 17 one derived from the intact chromosome 17 and two from the

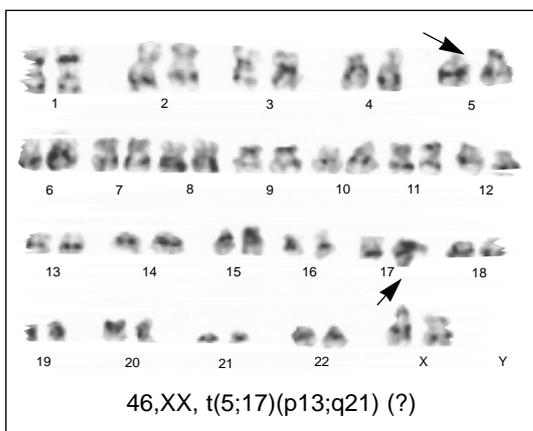


Figure 1. Karyotype from bone marrow: 46, XX,t(5;17)(p13;q21)?. Derivative chromosome 5 and 17 are shown by arrows. Chromosome 15 seemed to be normal by standart karyotype.

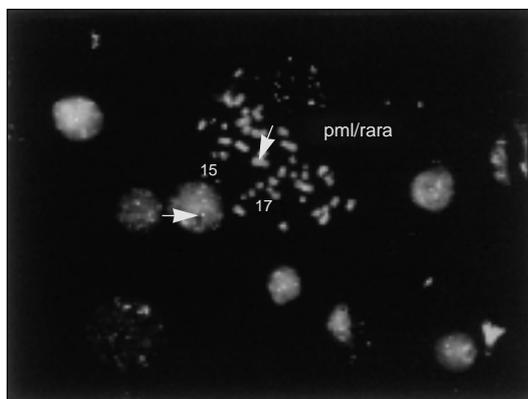


Figure 2. Fusion transcript PML/RARA by FISH: in an intephase cell and a metaphase (arrows): nuc.ish t(15;17)(PMLx1)(RARAx1)(RARAcnPMLx1) and ish. t(15;17)(PMLx1)(RARAx1)(RARAcnPMLx1).

rearranged chromosomes 15 and 17; for chromosome 15, one derived from the intact chromosome 15 and two from the rearranged chromosomes 15 and 17; for chromosome 5, one derived from the intact chromosome 5, and two from the rearrangements of chromosome 5 and 17 (Table 1). Due to chromosome arrangements size of the signals varied (Figs. 3,4,5).

The overall neoplastic karyotype after conventional cytogenetics and FISH studies were interpreted to be 46, XX, t (5;17) (p13;q22; q21).ish t(15;17) (PMLx1) (RARAx1) (RARA conPMLx1)

## DISCUSSION

The vast majority of cases of acute promyelocytic leukemia are associated with a specific translocation, t (15;17) (q22-q12-21). By conventional cytogenetics, the percentage of APL cases showing t (15;17) varies form 70% to 90%. At the molecular level this translocation results to the formation of some specific products namely as pml/rar-alpha, rar-alpha/pml and other aberrant pml products. In greater than 95% of APL cases the fusion product expressed is pml/rar alpha in which rar-alpha gene (retionic acid receptor alpha gene) on chromosome 17q is translocated to a putative transcription factor gene located on chromosome 15q known as pml. The formation of this fusion product pml/rara is suggested to be essential in the pathogenesis of APL and exists in al-

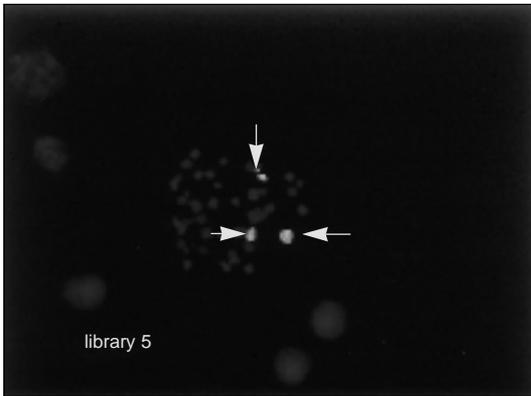


Figure 3. FISH by paint chromosome 5 showed: three chromosome 5 signals: bottom right, bottom left and up (smaller signal) paint five signals in a metaphase.

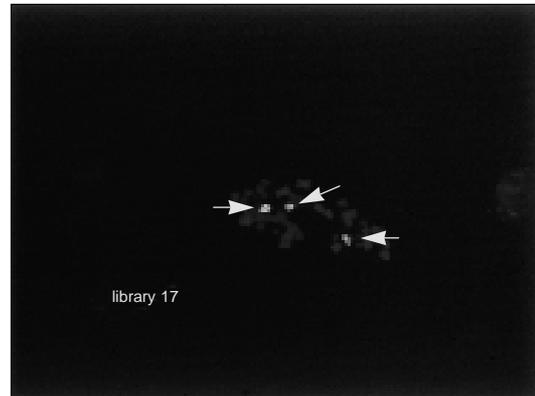


Figure 5. FISH by paint chromosome 17 showed: three chromosome 17 signals: right (smaller signal), middle and left in a metaphase.

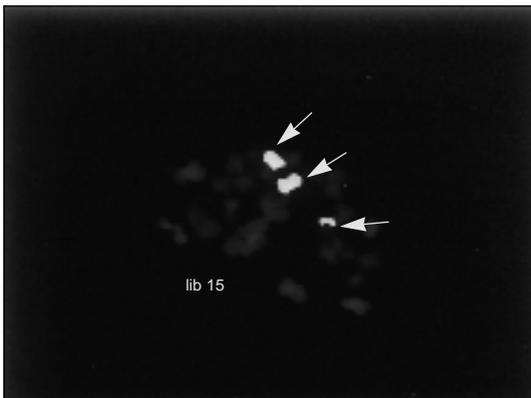


Figure 4. FISH by paint chromosome 15 showed: three chromosome 15 signals: up (two adjacent chromosomes) and bottom (smaller signal) paint fifteen signals in a metaphase.

es however a derivative 17q and 5p were detected. This rearrangement in association with the clinical data prompted us to go further by FISH analysis. Application of dual-colour FISH yielded clearly the specific pml/rar-alpha fusion in the cell nuclei as well as in the metaphases. In addition to that, FISH by paint probes 5,15 and 17 obviously showed that a complex translocation involving chromosomes 5,15 and 17, t (5;15;17) was present in this specific case of APL.

Translocations other than (15;17), namely variant translocations, are increasingly recognized in APL. In a few cases, rar-alpha variably translocates to chromosome 11 where it fuses to the PLZF gene or to a newly described partner NUMA and also to chromosome 5 where it fuses to the NPM gene (npm/rar fusion product)<sup>[10,14,15,17]</sup>. A second group in variant translocations in APL includes complex translocations occurring between 15, 17 and one or more other chromosomes. Approximately half of these cases show pml/rara fusion by molecular cytogenetic analy-

most all cases at the molecular level<sup>[1,4,6,8, 12,20]</sup>.

In the present study the common t (15;17) was not identified by conventional cytogenetic techni-

Table 1. Origin of three hybridization signals of chromosomes 5,15 and 17

Chromosome no.	Chromosome situation		
	Signal 1	Signal 2	Signal 3
15	intact 15	t(15;17)	t(17;15)*
17	intact 17	t(17;15)*	t(15;17)
5	intact 5	t(5;17)	t(17;5)*

\*The standart ISCN terminology is modified for better understanding of the situation

sis[9,12,20,21,22,23,24].

In APL as well as in many other leukemias, the presence of additional chromosome abnormalities has been shown to have adverse effect on prognosis. Additional therapeutic strategies may be required in patients with APL who demonstrate additional cytogenetic aberrations[2,3,9,13,19,22,23,24]. As a complex translocation existed in addition to pml/rara fusion in the present case, a possible poor prognosis was remarked. She received ATRA treatment in addition to cytostatic therapy. She showed a partial shortly durated remission but deceased soon after, in spite of the therapy. The cause of her death could not be evaluated as she was not in the hospital during that period.

APL has been shown to be the first model of differentiation therapy. Accurate diagnosis is now essential as all-trans retinoic acid therapy may mainly induce complete remission in patients whose leukemic cells harbour at (15;17) translocation resulting in pml/rara transcripts[5,6,7,14,16]. Furthermore the diagnosis of this useful marker, pml/rar alpha fusion, is also essential to monitor residual disease and the efficacy of ATRA in APL. Due to several technical problems the detection of the specific translocation, t(15;17) in APL patients, might yield serious difficulty by conventional cytogenetic investigations. Recent studies have proven that FISH analysis and RT-PCR are useful techniques as an adjunct to conventional cytogenetic approaches. Especially to provide a rapid and safe screening of putative residual cells, FISH and RT-PCR seem to be essential[7,10,19]. Nevertheless it is important to perform a conventional cytogenetic analysis because it enables the detection of additional chromosome aberrations which might imply prognostic significance.

The presence of additional chromosome abnormalities is identified in approximately 40% of APL patients and are presented to be important negative factors in the prediction of survival. However variant translocations are interpreted in a different attitude. There seems to be no major clinical distinction in between the cases with the classic t(15;17) and with those showing only variant translocations (6,18,23). Thus, it is important to make a differentiation between cases of APL showing t(15;17) at the molecular or cytogenetic level in association with secondary chromosomal

changes from those with only variant translocations. Our case presents a variant but complex translocation which has produced the essential pml/rara fusion transcript for the formation of APL but at the same time has produced additional chromosome rearrangements that could have served as a negative prognostic factor.

We believe that the present case is a good example for the impact of FISH in addition to conventional cytogenetic techniques in the diagnosis of APL in cases with no evident t(15;17). The technical approaches with respect to genetics support the idea that the key event in APL is the formation of the fusion gene PML/RARA on the derivative 15. The case also illustrates the usefulness of combined conventional cytogenetics and FISH technology for the detection of secondary chromosomal changes for the prediction of the response to treatment.

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