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CLINICAL CASES

Cytogenetic analysis and FISH of terminal deletion of the long arm of chromosome 9 in a patient with acute promyelocytic leukemia

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KEY WORDS
Promyelocytic Leukemia;
Terminal deletion;
Chromosome 9 del (9) (922).

Abstract
Deletions of the long arm of chromosome 9, del(9)(q22), are rare aberrations specifically found in acute myeloid leukemia (AML). Yamamoto *et al*, 1999, reported the first case of acute promyelocytic leukemia (APL) with a terminal 9q deletion as a sole abnormality. Here we describe the second case with the same aberration, the patient, an eleven-years-old girl with APL. Chromosomal analysis by the Giemsa R-banding technique and FISH using *BCR/ABL* and *PML/RARA* probe on short-term cell cultures from bone marrow was performed. A deletion of a 9 chromosome, del(9)(q22) was detected. Deletions of 9q have been described in about 3% to 4% of the AML patients, especially in M1 and M2 myeloid leukemia. Sole 9q terminal deletions, are less common than interstitial ones and involve q21~q22 band predominantly. A recent study suggests that 9q deletion, even in the absence of t(15;17), shows a relatively good prognosis. However, our patient died during the treatment.

PALABRAS CLAVE
Leucemia promielocítica aguda; Cromosoma 9 del(9)(q22); Deleción terminal.

Análisis citogenético y FISH de la deleción terminal del brazo largo del cromosoma 9 en un paciente con leucemia promielocítica aguda

Resumen
Las deleciones del brazo largo del cromosoma 9, del(9)(q22), son raras, y se observan específicamente en la Leucemia Mieloide Aguda (LMA). Yamamoto et al., 1999, publico el primer caso de leucemia promielocítica aguda (LPA) con un deleción terminal 9q, como la única aberración cromosómica presente. Aquí nosotros describimos el segundo caso con la misma aberración. El paciente, una mujer de 11 años de edad con LPA. El análisis cromosómico fue realizado en cultivos celulares de medula ósea usando la técnica de bandas R con Giemsa y FISH con sondas para detectar las fusiones BCR/ABL y PML/RARA. Fue identificada la deleción de una copia del cromosoma 9, del(9)(q22).

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Han sido descritas deleciones del brazo 9q en un ~ 3 a 4%, de pacientes con LMA, especialmente en los subtipos M1 y M2. La deleción terminal 9q, es menos frecuente que las deleciones intersticiales, y usualmente involucra las bandas q21~q22. Un estudio reciente sugiere que la deleción terminal 9q, aun en la ausencia de la traslocación entre los cromosomas 15 y 17, t(15;17), confiere relativamente un buen pronóstico. Sin embargo, nuestro paciente falleció durante el tratamiento.

Introduction

According to FAB classification (French - American - British cooperative group), acute promyelocytic leukemia (APL) is defined as an acute myeloid leukemia (AML) subtype M3.[1] APL is distinguished from other acute myeloid leukemias (AMLs) by cytogenetic, clinical, and also biological characteristics,[2-6] Although the basis of the diagnosis remains in the morphological characteristics, additional studies including immunophenotyping, cytogenetic evaluation, and molecular genetic analysis have become critical, and in some specific cases mandatory and complementary tools.[4]

APL typically carries a specific reciprocal chromosome translocation t(15;17) in the 95% of cases, leading to the expression of a leukemic-generating fusion protein, PML-RAR¤.[2,5,6] The APL molecular defect was found to be the disruption of the alpha receptor of retinoid acid (RAR¤) and its reciprocal, in frame fusion, with one of five partner genes: PML, located in chromosome 15q22, PLZF in 11q23, NuMA in 11q13, NPM in 5q32 and STAT5b in 17q11.2.[5] The cytogenetic abnormality in rare cases involves reciprocal translocations of chromosomes 5, 6, 7, 8, 11, 13, 19 and deletion (9q-).[5]

Terminal deletions of the long arm of chromosome 9, del (9q22-ter), are rare structural aberrations specifically found in AML.[7] Yamamoto et al, reported the first case of APL with a terminal 9q deletion as sole abnormality.[8] Here we describe the second case with the same structural aberration.

Case report

An eleven years old girl was admitted to the Pablo Tobón Uribe Hospital, Medellín, Colombia, in February 2000 with severe fatigue, and fever. Physical examination showed fever, tachycardia, pallor, weight loss, hepatomegaly and splenomegaly. Blood analysis revealed a hemoglobin level of 8.8g/dL, platelet count $12x10^9$/L, and white blood cells count of 55.2 x 10 9/ul with 12% neutrophils, 15% lymphocytes, and 71% blasts, diagnostic of acute leukemia. Examination of the bone marrow showed myeloid blasts (40%)

Figure 1. R-banded karyotype of the patient: 46,XX,del(9)(q22). The arrowhead indicates del(9)(q22)

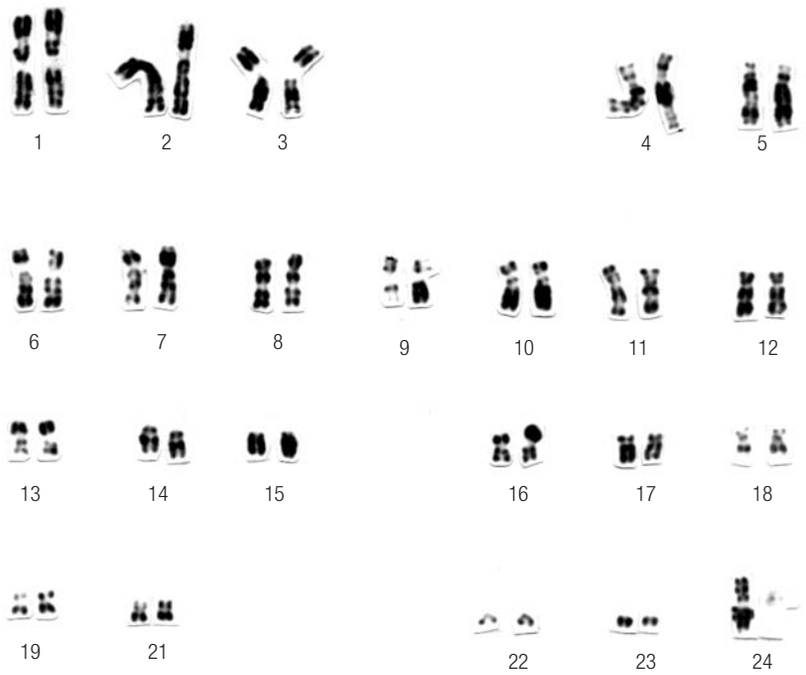


Figure 2. Fluorescence *in situ* hybridization analysis, using the dual –color, dual-fusion t(9:22) DNA probe. Locus Specific Identifier (LSI) *BCR* locus/22q11.2 (green signal), is present in the two normal chromosomes 22. LSI *ABL* locus /9q34 (red signal) is present in the normal chromosome 9. No red signal is detected in the chromose 9 with terminal deletion of the long arm

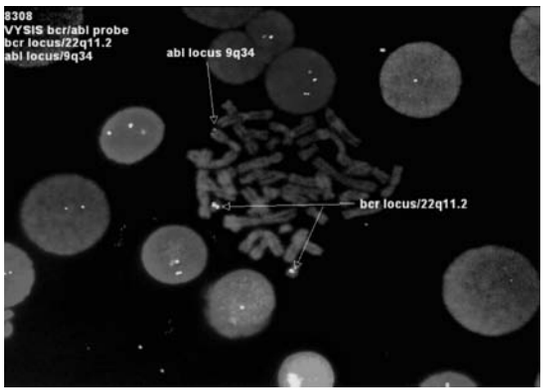
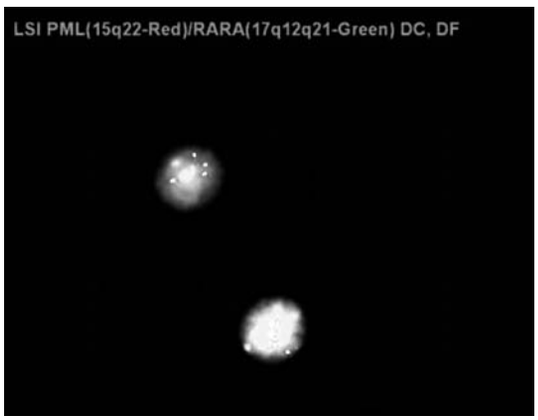


Figure 3. Fluorescence *in situ* hybridization analysis, using the dual-color, dual-fusion t(15;17) DNA probe. The cells in this image show the two orange (PML), two green (RARA) normal signal pattern. No fusion signals were detected



and atypical promyelocytes (35%). Cell suspension immunophenotypic studies were performed using CD3, CD13, CD19, CD33 and myeloperoxidase (MPO) reaction. Studies were positive for CD13, CD33 and MPO and negative for CD3 and CD19. According to FAB classification, a diagnosis of APL (AML M3) was made. Normal biochemistry liver and kidney was found. The patient was treated initially with cytarabine and mercaptanopurine. Four months after the diagnosis of APL, the patient was admitted to the Intensive Care Unit (ICU) with diagnosis of sepsis by *Escherichia coli* and secondary myocardial dysfunction. Finally, she died in ICU due to ventricular fibrillation.

Materials and methods

Chromosome analysis

Chromosomal analysis was performed on unstimulated 24-hours in vitro culture of a bone marrow specimen by the Giemsa R banding technique. Thirty metaphases and 300 nuclei were analyzed. Karyotype was described according to the International System for Human Cytogenetic Nomenclature.[9]

Fluorescence in situ hybridization (FISH) analysis

FISH to interphase nuclei and metaphase chromosomes was performed at the cytogenetics laboratories of Columbia University and UCLA, using a Locus Specific Identifier *BCR/ABL* Dual Color-Dual Fusion and *PML/RARA* Translocation Probe (Vysis, Inc.).

Results

Chromosome analysis

Chromosome analysis of bone marrow cells showed 46,XX,del(9)(q22.3) in all 30 metaphases (**figure 1**).

Fluorescence in situ hybridization (FISH) analysis

FISH analysis to interphase nuclei and metaphase chromosomes revealed del (9)(q22) as shown by the absence of one signal on chromosome 9 (**figure 2**).

FISH analysis to interphase nuclei revealed no *PML-RARA* rearangement (**figure 3**).

Discussion

In this study, R-banding and chromosome painting analyses showed both normal chromosome 9, and del(9)(q22). Deletions of the long arm of chromosome 9, del(9)(q22), are rare structural aberrations specifically found in acute myeloid leukemia (AML) as a sole chromosomal abnormality or as a secondary change, particularly together with translocations as t(8;21)(q22;q22).[10] Terminal deletions of 9q are less common than interstitial ones and involve 9q21~9q22 as observed in this case.[10] The most common breakpoints proximal and distal present in the interstitial deletions are 9q21 and 9q22 respectively.[11] Deletions 9q– occurs predominantly in APL subtype M1 and M2, but only seven cases of APL with 9q– deletions have been reported to date.[7] Yamamoto et al., reported the first case of APL with a terminal deletion of 9q– as sole chromosomal abnormality.[8] Our study is the second case with the same abnormality.

Chromosomal rearrangements in addition to t(15;17) have been reported in 25%-40% of APL.[3,12-19] The most frequent additional change was trisomy 8. Other abnormalities were far less frequent and usually involved chromosome 9, 17, 7, 21, 16, 6 and 12. The prognostic value of those additional cytogenetic abnormalities in APL patients remains controversial: Hiorns et al, found them to be associated with a poorer prognosis, Schoch et al, and Grimwalde et al., found them to be associated with a similar prognosis and Slack et al, with a slightly better prognosis.[10,14,16,19]

Table 1. Reported cases of APL with 9q deletion

Age/Sex	Karyotype	Survival (weeks)	References
38/M	46,XY,del(7)(q22),del(9(q22),t(15q+; 17q_)	8	12
22/M	46,XY,del(9)(q22?),t(15q+;17q-)	4	12
50/F	46,XX,del(9)(q22?),t(15q+;17q-)	1	12
30/M	46,XY,del(9)(q21q33?),t(15q+;17q-)	9a	12
NA	46,X?,del(9)(q?), t(15;17)	NA	21
59/M	46,XY,del(9)(q11q23),t(15;17)(q22;q21)	NA	22
38/M	46,XY,del(9)(q1?q22),t(15;17)(q22;q21)	NA	22
25/F	46,XX,del(9)(q22)	2	8
11/F	46,XX,del(9)(q22)[30]	16	Present case

NA: not available
[a]Survival from chromosome analysis at relapse

As shown in **table 1**, nine cases of APL with 9q – have been reported.[8] All but the last two cases had t(15;17) and five of them had terminal deletions. No specific morphological feature has been reported in APL with 9q–.[20] Survival in five of these cases seems to be very short, although four cases were treated without ATRA. FISH performed in our patient with a DNA probe flanking the breakpoints of t(15;17) did no show the retinoic acid receptor alpha (RARA*)/PML fusion signal usually generated on the der(17)t(15;17) (**figure 3**). This result does not exclude the diagnosis of APL. It is possible that this patient had a cryptic chromosome rearrangement undetectable by karyotype and FISH analysis. This is known to occur in approximately 1% of APL cases.[21] In such cases, reverse-transcriptase PCR, RT-PCR, is the best test to perform with a detection sensitivity of ~1 in 10^5 cells compare to the 1% sensitivity of FISH. Unfortunately, RT-PCR analysis was not performed in our patient. At the time of diagnosis this test was not available for clinical diagnosis. Nowadays, it is widely available, not only to identify a PML-RARA fusion due to a cryptic rearrangement in patients with flow cytometric and bone marrow morphology features characteristic of APL but also to follow up patients for minimal residual disease.[22] A fresh sample for RNA extraction is needed to perform the RT-PCR, however, this is not available in our case.

The present patient did not respond to chemoterahpy with cytarabine and mercaptanopurine and died in the course of the treatment four months after the diagnosis of APL. Therefore, our report suggests that 9q– may also be an adverse prognostic factor in APL subtype AML, M3. More cases need to be investigated to elucidate the precise role of 9q– in the pathogenesis of APL and its clinical significance.

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