Table 1. Baseline demographic characteristics and mean values for the three hypercoagulative markers in the patients and in the control subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hip</th>
<th>Knee</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>53</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>64</td>
<td>67</td>
</tr>
<tr>
<td>Percentiles 25-75%</td>
<td>60-73</td>
<td>64-70</td>
<td>62-73</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>26</td>
<td>23</td>
</tr>
</tbody>
</table>

Indication for surgery *(more than one diagnosis could be present in the same patient)*
- Osteoarthritis: 39
- Necrosis: 10
- Rheumatoid arthritis: 7
- Miscellaneous: 2

Markers
- Mean
  - D-D (ng/mL): 1,135.5
  - TAT (µg/L): 5.3
  - F1+2 (nmol/L): 1.7
- Percentiles 25-75%
  - D-D (ng/mL): 665.14-1,339.43
  - TAT (µg/L): 2.1-7.6
  - F1+2 (nmol/L): 1.4-2.1

**Keys words**
- Hypercoagulability, hip and knee arthroplasty, venous thromboembolic disease

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**References**

Cryptic insertion (15;17) in a case of acute promyelocytic leukemia detected by fluorescence in situ hybridization

Sir,

We report the case of a patient with acute promyelocytic leukemia (APL) with no detectable cytogenetic abnormalities. Fluorescence in situ hybridization (FISH) studies demonstrated an insertion of the RARα gene into one copy of chromosome 15. RT-PCR studies showed a PML/RARα transcript. The patient achieved complete remission with chemotherapy and ATRA, but relapsed during maintenance therapy with ATRA.

Acute promyelocytic leukemia (APL) is characteristically associated with the reciprocal chromosomal translocation t(15;17)(q22;q21) which is identified in up to 90% of cases by conventional cytogenetics. However, a few cases with submicroscopic rearrangements of RARα gene have been described.1

A 27-year-old man was admitted to our hospital because of a one-week history of weakness and fever. Blood cell count showed: Hb 79 g/L; WBC 45.9×10⁹/L with 79% hypergranular blast cells and platelets 39×10¹⁰/L. The bone marrow findings were consistent with classical APL (AML-M3) according to the FAB criteria. The immunophenotype showed: CD13+, CD33+, HLA-DR+ and CD45a+. He was treated according to the European APL/93 protocol (ATRA in combination with cytosine arabinoside and daunorubicin) and achieved a complete remission on day 30 of treatment. The patient relapsed, 20 months after diagnosis, during maintenance therapy with ATRA. A second remission was obtained with Ara-C, mitoxantrone and etoposide. Afterwards, he received an allogeneic peripheral blood stem cell transplantation (PBSCT) from his HLA-identical sister. The patient developed a veno-occlusive disease and acute graft-versus-host dis-
ease and died on day 40 after PBSCT.

**Cytogenetics:** at the time of diagnosis and relapse, bone marrow samples were cultured for 48 hours according to standard procedures. A normal karyotype was observed in the 20 metaphases examined. **FISH:** two-color FISH was performed using painting probes for whole chromosomes 15 (Cambridge, Cambridge, UK) and 17 (Oncor, Gaithersburg, MD, USA) and revealed two intact copies of both chromosomes in the 35 metaphases analyzed. An APL t(15;17) translocation probe (Vysis, Stuttgart, Germany) demonstrated the presence of the PML/RARα fusion gene on one copy of chromosome 15 (Figure 1).

**RT-PCR:** in vitro reverse transcription (RT) of 1 µg of total RNA to cDNA and RT-PCR amplification of PML/RARα and RARα/PML transcriptions were performed using standard methods (GeneAmp RNA PCR kit; Perkins Elmer-Cetus, Norwalk, CT, VSA). A PML/RARα transcript of the bcr-1 type (DNA fragment of 326 bp) was observed, however the reciprocal RARα/PML transcript failed to be amplified.

This report describes an interstitial insertion of RARα gene from chromosome 17 into the PML gene on chromosome 15 in an APL patient with an apparently normal karyotype. The cryptic PML/RARα rearrangement was detected by FISH with an APL t(15;17) probe and was confirmed by RT-PCR, which showed the presence of a hybrid PML/RARα transcript but not of the reciprocal RARα/PML transcript. A number of variant translocations associated with APL including submicroscopic translocations have been described. However, the characterization by FISH of cases with cryptic PML/RARα rearrangements in apparently normal chromosomes 15 and 17 is unusual. The presence of the PML/RARα fusion gene determines the sensitivity to ATRA treatment, while the cytogenetic variants of APL not leading to a PML/RARα fusion, for instance t(11;17) and t(5;17), fail to respond to ATRA. Although the molecular consequences of this interstitial insertion are apparently identical to those observed in the classic RARα rearrangement, the molecular mechanisms should be different since another chromosome break distal to RARα has been produced to allow the insertion. Whether or not this different molecular mechanism implies a different clinical course and an unfavorable prognostic factor which could be related to the relapse of the patient during maintenance therapy with ATRA needs to be clarified.

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**Key words**

Acute promyelocytic leukemia, insertion (15;17), cytogenetics, FISH.

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**References**

C→T mutation at -158 8γ HPFH associated with 4 bp deletion (–225–222) in the promoter region of the 8γ gene in homozygous β^0 39 nonsense thalassemia

Sir,

Two Caucasian brothers from Central Spain were found to have homozygous β^0 thalassemia with mild anemia and mild physical stigmata of thalassemia. Molecular studies revealed that both subjects were homozygotes for the nonsense mutation of codon 39 (C→T), and heterozygotes for the C→T mutation at position –158 to the 8γ gene [Xmn I-γ (+)] and for the 4 bp deletion (–225–222) in the promoter of the 8γ gene.

β-thalassemias are a heterogenous group of genetic alterations characterized by a deficient synthesis (β+) or an absence (β-0) of β globin chains. The clinical expression of this disease can range from asymptomatic cases in most heterozygote forms of β-thalassemia (thalassemia minor) to severe forms of the disease (thalassemia major) in which the patients, usually homozygotes or double heterozygotes, are transfusion dependent. However, between these two extreme clinical forms there are a wide range of clinical phenotypes.

We have studied two Caucasian brothers, 26 (II1) and 31 (II2) years old, from Central Spain. Physical examination revealed normal body structure with a splenomegaly of 5 cm in II1 and 6 cm in II2, and mild signs of thalassemic facies and conjunctival jaundice in both. Their father (I1) and mother (I2) were not related but both had thalassemia minor. The subjects had a more severe phenotypic expression than their parents with mild anemia (Table 1).

Both subjects were homozygotes for the nonsense mutation of codon 39 (C→T) and their parents were heterozygotes for this mutation (Figure 1). This mutation produces a lack of expression of the β gene (β-0) and has been reported to be responsible for thalassemia major. The existence of α-thalassemia, which would have produced a less pronounced phenotypic expression of the disease, was ruled out by Southern blot analysis with Bam HI, Bgl II, Hph I, Nco I and Eco RI restriction enzymes and α and ζ probes.

In the last decade some forms of non HPFH-deletion, which can “improve” the expression of the disease, have been described. These forms are due to point mutations of one base upstream of the 8γ or 8γ gene. Most of these mutations are associated with levels of Hbf from 5 to 25% in heterozygotes and levels of Hbf greater than 5% when associated with heterozygote β-thalassemia. In the two cases reported here the parents are carriers of heterozygous β-thalassemia and the levels of Hbf are lower than 3% in both (Table 1). On the other hand, the substitution C→T at position –158 of the 8γ gene [Xmn I-γ (+)] is associated with increases in Hbf in situations of severe anemia and stress erythropoiesis (homoygote SS, homozygote or double heterozygote β-thalassemia) which would result in a decrease in the clinical severity of these situations. However, these Xmn I-γ (+) are not associated with a significative increase in Hbf in normal individuals or heterozygote β-thalassemia. The molecular studies revealed that the mother and the two brothers had the C→T mutation at position –158 to the 8γ gene [Xmn I-γ (+)] in the heterozygote form (Figure 1). This finding could explain the clinical picture of the disease, with a mild anemia of 10.5 to 11.5 g/dL of Hbf and a 8/8γ ratio of 2:1, higher than the expected 2:3, in the brothers, and a Hbf level less than 3% in the mother who has heterozygote β-thalassemia. Other forms of non HPFH-deletion are associated with levels of Hbf greater than 5% when associated with heterozygote β-thalassemia. In this context, the presence of another form of non HPFH-deletion associated in this family is not probable.

At the level of the promoter of the gene 8γ both the brothers and the parents had a 4 bp deletion (–225–222) (Figure 1). This deletion of 4 base pairs is associated with a 8γ expression of greater than 5% when associated with heterozygote β-thalassemia (Table 1).

Table 1. Hematologic values and biochemical studies.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>I1 (father)</th>
<th>I2 (mother)</th>
<th>II1</th>
<th>II2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC × 10^12</td>
<td>6.9</td>
<td>6.3</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>41.6</td>
<td>38.1</td>
<td>33.7</td>
<td>31.4</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.6</td>
<td>12.5</td>
<td>11.3</td>
<td>10.6</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>59.2</td>
<td>61</td>
<td>75</td>
<td>67.6</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.6</td>
<td>20</td>
<td>25.5</td>
<td>22.8</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>33.1</td>
<td>32.8</td>
<td>33.6</td>
<td>33.8</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.5</td>
<td>14.9</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>6.2</td>
<td>5.9</td>
<td>7.4</td>
<td>8.3</td>
</tr>
<tr>
<td>IMR: (MFR+HFR)×100/LFR</td>
<td>3.5</td>
<td>6.9</td>
<td>13.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Hb A2 (%)</td>
<td>5</td>
<td>5.2</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>2.9</td>
<td>2.3</td>
<td>97.5</td>
<td>96.9</td>
</tr>
<tr>
<td>aHb /aHb</td>
<td>-</td>
<td>-</td>
<td>2/1</td>
<td>2/1</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>-</td>
<td>-</td>
<td>226</td>
<td>186</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>-</td>
<td>4.8</td>
<td>3.4</td>
<td>3.4</td>
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<tr>
<td>Serum iron (g/dL)</td>
<td>-</td>
<td>171</td>
<td>108</td>
<td>108</td>
</tr>
<tr>
<td>TIBC (g/dL)</td>
<td>-</td>
<td>-</td>
<td>193</td>
<td>184</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>-</td>
<td>386.2</td>
<td>454.8</td>
<td>454.8</td>
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