

## ACUTE PROMYELOCYTIC LEUKEMIA WITH UNUSUAL MORPHOLOGICAL FEATURES, ABSENCE OF THE t(15,17) AND REARRANGED PML/RAR $\alpha$ GENES

*Angelo Cantù-Rainoldi\**, *Augusto Bramante Federici\*\**, *Andrea Biondi*, *Agostino Cortelezzi°*, *Lorenza Romitti\**, *Gianluca Gornati\**, *Giuseppe Gaipa\**, *Gianangelo Gianotti\**, *Piermannuccio Mannucci\*\**

*Laboratorio di Ricerche Cliniche, Anatomia ed Istologia Patologica e Centro Trasfusionale, Istituti Clinici di Perfezionamento, Milano; Centro Emofilia e Trombosi Angelo Bianchi Bonomi ed Istituto di Medicina Interna ed °Istituto di Scienze Mediche, IRCCS Ospedale Maggiore ed Università di Milano; Clinica Pediatrica dell'Università di Milano, E.O.G. Monza, Italy*

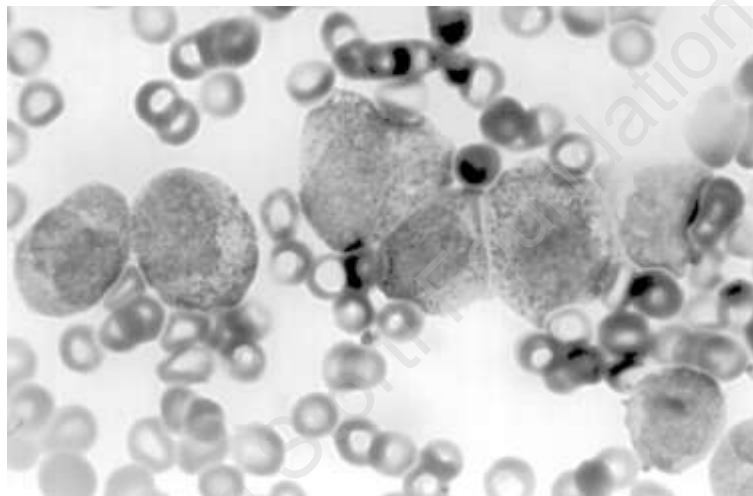


Figure 1. Bone marrow, MGG staining (x1000).

**A**cute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia with unique morphologic, cytogenetic, molecular and clinical features. Besides the classical hypergranular variant, there is also a cytological microgranular variant with significant clinical implications in both children<sup>1</sup> and adults.<sup>2</sup> Moreover, a basophilic form<sup>2,3</sup> and a hyperbasophilic microgranular variant<sup>4</sup> have been described, suggesting further morphological heterogeneity.

t(15;17) is considered the specific cytogenetic

marker for both the hypergranular and microgranular variants, and diagnosis of APL could be questionable in the absence of this cytogenetic abnormality. PML and RAR $\alpha$  genes are involved by t(15;17) and their rearrangement is considered the molecular counterpart of the translocation.<sup>5</sup> However, the existence of cases lacking this translocation but showing rearrangements of the PML/RAR $\alpha$  genes has recently been reported.<sup>6</sup> The availability of a more sensitive approach to APL diagnosis would obviously be of great importance in view of the thera-

peutic protocol which presently includes the use of transretinoic acid (T-RA), a differentiating agent that induces a complete remission in a large percentage of APL patients.<sup>7</sup>

In this paper we describe a case of APL with a normal karyotype but rearranged PML/RAR $\alpha$  genes and an unusual morphologic picture.

### Case report

The patient is a 39-year-old man who came to our observation in October, 1993 because of sudden, serious gum bleeding. Thrombocytopenia (plt  $32 \times 10^9/L$ ) and mild anemia (Hb 13.2 g/dL) were found upon analysis, and granulated blasts were observed in the blood smear. Total white cell count was  $5.6 \times 10^9/L$ . Blood coagulation tests were suggestive of decompensated DIC: prolonged prothrombin time (PT; ratio 1.75) and partial thromboplastin time (PTT; ratio 1.27), low fibrinogen levels (66 mg/dL) with increased fibrin degradation products (500-1000  $\mu\text{g/mL}$ ). The patient was immediately hospitalized and treated with 2 U of fresh frozen plasma (FFP). Needle bone marrow aspiration was successfully performed and samples were collected for morphology, immunophenotyping, cytogenetics and molecular biology. Although preliminary diagnostic data were not completely probative (see results in detail), the patient underwent treatment for APL according to the suggestions of the GIMEMA/AIEOP group, which include the use of T-RA (45 mg/sqm per os daily from diagnosis to day 60) and idarubicin (12 mg/sqm on days 2, 4, 6, 8). The patient tolerated therapy very well without any complications, recovered quickly from the aplastic phase receiving only units of FFP and platelet concentrates, and did not show any opportunistic infections. He was dismissed from the hospital one month later and since then has been followed every week. At the end of the 60-day treatment with T-RA, the blood picture was normal and bone marrow aspirate showed morphologic remission. After achievement of remission, two more cycles of consolidation were administered according to the protocol.

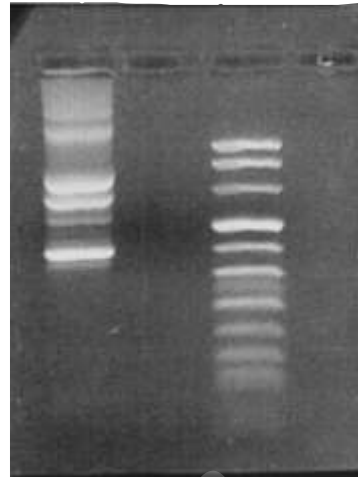


Figure 2. Detection of PML/RAR $\alpha$  fusion by PCR in a BM sample at diagnosis (lane 1). The ethidium bromide-stained gel containing bands corresponding to the BCR-1 PML/RAR $\alpha$  junction is shown.

### Morphological, cytogenetic and genotypic features

Atypical cells in both the peripheral blood and in the bone marrow showed numerous azurophilic granules that were not as strictly arranged as in typical hypergranular APL, giving them the appearance of cells at an intermediate stage of differentiation between myeloblasts and promyelocytes. There were no Auer rods and cells were larger than those usually seen in APL, and nuclei were eccentrically placed (Figure 1). Myeloperoxidase was strongly positive in the whole population and aspecific esterases ( $\alpha$ -naphthyl-acetate esterases, ANAE) were negative. The immunophenotype showed the typical APL pattern (HLA-DR $^-$ , CD13 $^+$ , CD33 $^+$ , CD9 $^+$ , CD11b $^-$ , CD14 $^-$ , CD34 $^-$ , CD7 $^-$ , CD5 $^-$ , CD2 $^-$ , TdT $^-$ ).<sup>8</sup>

Cytogenetic analysis was performed on both peripheral blood and bone marrow samples. Cells were incubated in RPMI 1640 supplemented with 20% fetal calf serum without mitogens for 48 hrs. A total of 37 metaphases, 10 from PB and 27 from BM, was scored by means of QFQ banding. A normal 46,XY karyotype was detected in all the cells examined.

Reverse transcription-PCR (RT-PCR) analysis to detect the presence of PML/RAR $\alpha$  mRNA was carried out as previously described and

revealed the presence of the PML/RAR $\alpha$  chimeric product resulting from a *BCRr-1* type PML breakpoint.<sup>9</sup>

Molecular genotyping, performed by polymerase chain reaction (PCR) analysis of PML/RAR $\alpha$  transcripts, demonstrated the presence of PML/RAR $\alpha$  rearrangement (BCR-1) (Figure 2).

### Conclusions

We describe an unusual case of APL with a morphologic picture resembling that of a hypergranular form but with some cytological peculiarities, such as fewer granules, the absence of Auer rods and bigger cell size. In spite of strong myeloperoxidase expression and an immunophenotype suggestive of APL, diagnosis would have been questionable in the absence of molecular analysis because of the atypical morphology and the normal karyotype. A more precise definition of the cytological spectrum of APL is definitely needed, considering the use of T-RA in most of the ongoing APL protocols. As is known, APL is easily diagnosed in the typical hypergranular cases, but it may be difficult to recognize the microgranular variant<sup>10</sup> and the atypical cases like the one reported in the present paper. It would be of interest to explore whether any differences exist at the molecular level among the different morphologic APL variants.

In summary, we believe that the cytological heterogeneity of APL should be considered in view of the important therapeutic implications

related to such a diagnosis; molecular analysis may represent an important tool for identifying previously unrecognized cytological APL variants and for confirming the therapeutic approach with T-RA in doubtful cases.

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