

Acute promyelocytic leukemia microgranular variant – A clinical and therapeutical approach

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ABSTRACT

Acute promyelocytic leukemia (APL) is a rare form of acute myeloid leukemia with a particular diagnosis and therapeutic approach. APL represents only 10-15% of acute myeloid leukemia in adults. The median diagnosis age is around 40 years. APL's main genetic event is the translocation between chromosome 15 and chromosome 17 – t(15;17)(q22;q21) – generating the PML-RAR α fusion gene. APL has four different subtypes: the classic form M3 hypergranular, the microgranular variant, the hyperbasophilic form and zinc-finger form, recently described, which is characterized by the presence of a different translocation, between chromosome 11 and chromosome 17: t(11,17)(q23, q11-12). Clinically, patients associate hemorrhagic manifestations and life threatening coagulopathy.

Secondary acute myeloid leukemias constitute an entity with a growing incidence in the last few years due to the leukemogenic effect of radiotherapy and several antineoplastic agents for solid tumors, even if administrated at low doses (4).

We chose to report the case of a 67 year-old woman diagnosed with APL-variant type, who has a history of breast cancer treated by surgery, chemo- and radiotherapy twenty years before the leukemia was diagnosed.

Keywords: acute promyelocytic leukemia, PML/RAR α , ATRA, complete molecular remission.

INTRODUCTION

Acute promyelocytic leukemia, a rare disease, requires special attention among the subtypes of AML for several important reasons. APL represents approximately 10-15% of AML in adults. The median age at the time of the diagnosis is around 40 years, which

means that the patients are considerably younger than in the other subtypes of AML (70 years). The disease may be the consequence of prior therapy for an unrelated malignancy; in these cases, the prognosis is as good as the one for the individuals who present with de novo disease, in contrast with other subtypes of AML in which the prognosis is significantly worse (1,2).

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APL is due to a translocation between chromosome 15 and chromosome 17, which is the result of two chromosome breaks. The break in chromosome 15 disrupts the promyelocytic leukemia (PML) gene which encodes a growth suppressing transcription factor. The break in chromosome 17 interrupts the retinoic acid receptor alpha (RAR α) gene which regulates myeloid differentiation. The translocation creates the PML/RAR α fusion gene. It produces a chimeric protein which arrests the maturation of myeloid cells at the promyelocytic stage.

APL has four different subtypes: the classic form M3 hypergranular, the microgranular variant, the hyperbasophilic form and the zinc-finger form, recently described, which has a different translocation, between chromosome 11 and chromosome 17 – t (11,17)(q23, q11-12) (3,4).

The characteristic clinical features of APL include hemorrhagic manifestations and life threatening coagulopathy.

The treatment of APL has dramatically changed with the discovery of the activity of all trans-retinoic acid (ATRA) in this disease (1,5). APL has become the most curable of all acute myeloid leukemia subtypes, by using the current therapy, including ATRA and anthracycline-based chemotherapy and maintenance therapy with ATRA (6,7,8). ATRA therapy has one serious and specific complication: retinoic acid syndrome (RAS). RAS is characterized by signs and symptoms: fever, dyspnea, leukocytosis, pulmonary infiltrates, pleural or pericardial

effusions, hypotension, renal dysfunction (9,10). The median time of onset is 7 days after ATRA treatment has begun. High dose dexamethasone has proved efficacy in RAS management.

In patients who relapse, treatment may include arsenic trioxide, monoclonal antibodies, bone marrow transplantation (11,12,13,14,15). \square

FULL CASE STUDY

The patient is a 67-year-old woman who is hypertensive, dyslipidemic, with a history of breast cancer treated by surgery, chemo- and radiotherapy (1988), myocardial infarction (2001), with early angina pectoris; she was in her usual state of health until 2 weeks prior to presentation (September 2006), when she began to complain of fatigue, gingival bleeding, epistaxis, and easy bruising.

The physical examination revealed pallor, few ecchymotic areas on the upper and lower extremities, bleeding gums. No lymphadenopathy or hepatosplenomegaly were noted. Vital signs were stable.

CBC showed: WBC 38,600/ml, hemoglobin (Hb) 7.9g/dl, hematocrit (Ht) 23.2%, platelet count 22,000/ml. \square

Morphology

On peripheral smear: 65% atypical promyelocytes with folded nuclei, few granules and Auer rods (Figure 1,2).

The **bone marrow** aspirate revealed hypercellularity, with 88% atypical promyelocytes with folded nuclei, basophilic cytoplasm and few with granules and Auer rods.

Immunophenotype: the flow cytometric examination revealed that the blasts were CD33+, CD34+, CD65w+, CD117+/-, CD2 low+, CD56-/+ HLA-DR low+ and negative for CD14, CD15 (Figure 3) (16).

At the time of the diagnosis, it was also collected a peripheral blood sample for **molecular detection** (Reverse transcriptase polymerase chain reaction) of PML/RAR α , which revealed the presence of the molecular genetic marker of APL (17). It was used the quantitative RT-PCR method (26).

In this particular case, the laboratory tests were negative for disseminated intravascular coagulation (normal PT, INR, APTT, fibrinogen level, D-Dimers), otherwise frequently present at diagnosis. \square

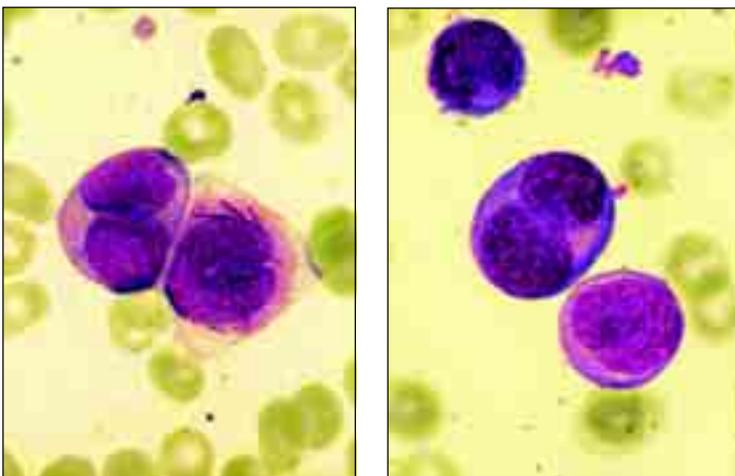


FIGURE 1 and FIGURE 2. Review of the peripheral smear confirmed the presence of abnormal promyelocytes, with folded nuclei, irregular borders, less prominent granules, few with Auer rods.

Diagnosis

Based on the available data, the diagnosis of acute promyelocytic leukemia (APL) – microgranular variant – was established. The positive diagnosis was suggested by the clinical features (hemorrhagic findings), and the characteristic morphologic findings on peripheral blood and bone marrow smears examination. Flow cytometry (18) and reverse transcriptase polymerase chain reaction were also performed in order to confirm the diagnosis and to allow a differential diagnosis from acute monocytic leukemia.

The **differential diagnosis** (19) of APL microgranular variant includes the classic form M3 hypergranular.

Differential diagnosis of the microgranular variant also includes other acute non-lymphoid leukemia, monocytic subtypes (AML4, AML5).

The promyelocytic cells in microgranular variant may sometimes be mistakenly identified as being of monocytic origin, based on the features of the blasts, especially the folded nuclei. The presence of the Auer rods in the microgranular variant and their absence in monoblasts, as well as the lack of granules, make the difference between the two forms.

Cytochemistry (**Figure 4**): promyelocytic cells are positive for myeloperoxidase, while monocytic blasts show non-specific esterase activity.

Flow cytometry (**Figure 3**): the presence of myeloid lineage markers (CD33, CD65w) and the absence of monocytoid lineage antigens (CD14 and CD64 high positive). In this particular case we found some aberrant expressions of myeloid markers for a promyelocytic acute leukemia, i.e. CD117 (c-kit), CD34 and lack of CD15, that suggest an immature phenotype, frequently associated with microgranular variant

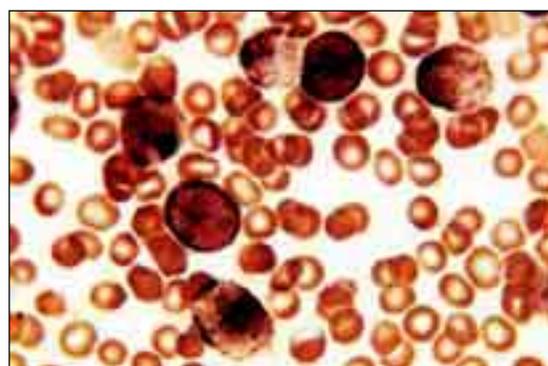
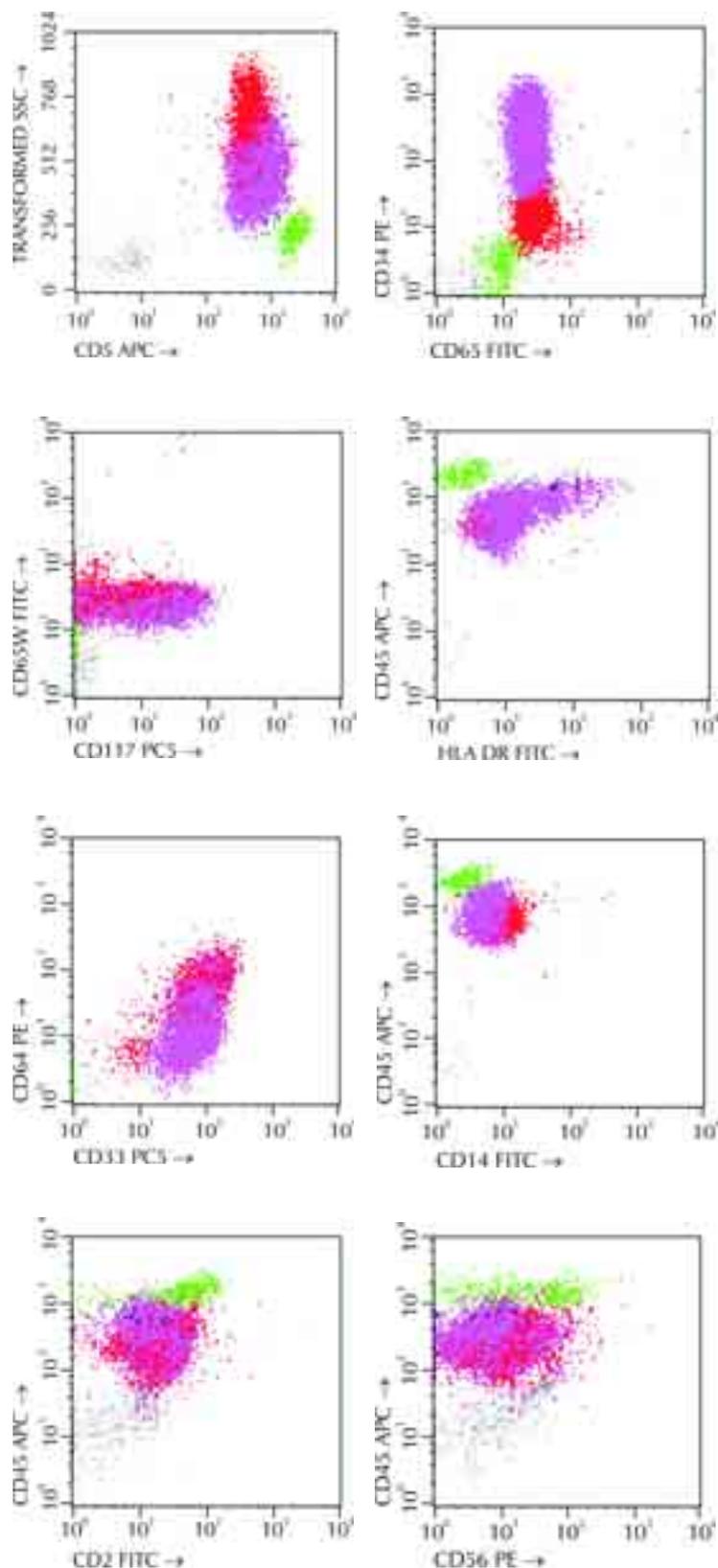


FIGURE 4. Peroxidase stain on bone marrow aspirate in M3 variant AML

FIGURE 3. Dot-plot histograms in APL: *Atypical Promyelocytes* (colored in violet) with the following profile: CD33+ CD65w+ CD34+ CD117+/- CD64low+ CD2low+ HLA-DR low+ CD56-/+ CD14- CD15-. FACS-Calibur acquisition, CellQuest software.

(27). The expression of HLA-DR is also low positive, not usual for this diagnosis, but it was reported in some papers. Low positivity expression of CD2 suggest microgranular variant, also (28). □

Prognosis

At diagnosis we identified features that predict a high risk for relapse; the old age, elevated WBC at presentation, low platelets count.

The morphological subtype, microgranular variant, and the onset hyperleukocytosis are associated with higher incidence of RAS and DIC, increasing the early mortality and morbidity.

The patient has a poor prognosis because of associated cardiac pathology that limits the therapeutic possibilities (anthracyclines used in induction and consolidation therapy have cardiac toxicity). □

Treatment

The treatment goal in APL patients is to achieve a complete molecular remission, defined by the absence of the PML/RAR α transcript using RT-PCR methods.

The patient underwent the classic induction therapy with all-trans-retinoic acid and anthracyclines (6,7,8). APL is the first known disease that is clinically sensitive to differentiation therapy. ATRA given as a single therapeutic agent is associated with short remissions and relapse; that is why combined chemotherapy (ATRA with conventional chemotherapy) is recommended, with prolonged disease-free survival (20, 21, 22, 23, 24, 25).

The patient received induction therapy with mitoxantrone (10mg/m²/day, for three days), ATRA (45mg/m²/day) and low doses of dexamethasone for ATRA syndrome prophylaxis. Mitoxantrone was used instead of daunorubicin, because of the lower cardiac toxicity. On the third day of the treatment, the patient developed hyperleukocytosis, respiratory distress, radiographic pulmonary infiltrates, and episodic hypertension, without renal failure. RAS was blocked by treatment with high doses of dexamethasone (10 mg iv every 12 hours), oxygen, diuretics (9,10). Favorable evolution was noted. The developed leukocytosis while receiving ATRA was treated with hydroxyurea 2.5g/day, hydration and allopurinol. Because of the leukemic medullar infiltration and

chemotherapy, the patient required the usual blood product support and antimicrobials. The high dose dexamethasone treatment induced high levels of glucose that necessitated insulin therapy.

Complete hematological response was achieved after induction course and confirmed by bone marrow aspiration. After receiving one course of induction chemotherapy, the patient was subjected to consolidation therapy, first cycle with idarubicin (5mg/m²/day for 4 days) and ATRA (45mg/m²/day), the second one with mitoxantrone (10mg/m²/day for 5 days) and the third one with idarubicin (5 mg/m² for a single day).

At the end of the consolidation courses, a follow up study of the molecular response was recommended and it confirmed the molecular remission (PML-RAR α transcript was negative).

The maintenance therapy recommended was: ATRA 45mg/m² for 15 days/quarterly in association with Methotrexate 15mg/m² weekly and Mercaptopurine 50mg/m² daily for one year. □

Treatment monitoring

Molecular monitoring of the PML/RAR α status while maintenance therapy may predict the relapse risk and permit early therapeutic intervention before overt clinical relapse occur. RT-PCR for PML/RAR α should be performed every three months for the first 2 years and every six months for the following 2-3 years (17). □

Conclusion

Acute promyelocytic leukemia (APL) is a biologically and clinically distinct type of acute myeloid leukemia. Sometimes it can occur after chemotherapy for another cancer, especially after use of topoisomerase II inhibitors. In contrast to other AML types this is not a predictor of poor prognosis in APL. The patient presented is a 67 years woman with secondary acute promyelocytic leukemia microgranular variant that is probably related to treatment for breast cancer. Immunophenotypic profile was atypical for APL, with immature markers and correlate to microgranular variant. She experienced a good outcome with persistent complete molecular remission, despite the initial poor prognosis factors.

The absence of disseminated intravascular coagulation, a frequent complication in acute promyelocytic leukemia at onset was a particular feature of the case. □

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