ACUTE PROMYELOCYTIC LEUKEMIA IN FOUR YEAR-OLD FEMALE CHILD, A CASE REPORT

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ABSTRACT

30% of pediatric oncohematologic malignancy are acute leukemias and in this area the AML incidence is very low, much less than the acute promyelocytic leukemia. We report the case of a 4 year old girl with acute promyelocytic leukemia whose blasts showed the morphology characteristic of acute promyelocytic leukemia variant and the Fluorescence In Situ Hybridization confirmed the presence of t(15;17) translocation. The case is reported because in the pediatric population the acute promyelocytic leukemia is a rare occurrence; moreover, it represent a true oncohematologic emergency, in this case the laboratory has a significant role since the timing of diagnosis must be very short.

Key words: Acute Promyelocytic Leukemia, t(15;17) traslocation, ATRA, ADVIA 2120i (Siemens Healthcare ®) blood counter analyzer, cytograms

INTRODUCTION

Leukemia is the most common malignancy of childhood representing about 30% of oncohematological diseases diagnosed in children under 15 years of age. In childhood most cases of leukemia are acute leukemia and are characterized essentially by the lymphoid B-population involvement 1.

Acute myeloid leukemia (AML) in pediatric field represent the 15-20% and the related mortality is approximately 30%. AML incidence in children is about 5-7 cases per million children year, the incidence peak is 11 cases per million in children under two years of age 2-3. AML incidence is very low at around 9 years of age, during adolescence it is 9 cases per million approximately, this data remains stable until about 55 years of age 4. With regard gender and race, black or white, there is no difference, however, AML
incidence appears to be higher in Hispanic children. Between pediatric AML the incidence of acute promyelocytic leukemia (APL) is <10%\textsuperscript{5}. A high frequency of APL, between 25-59%, has been reported in South and Central America\textsuperscript{6-7}; in Latino (Brazilian) pediatric patients, it could be related to ethnic background and/or exposure to different environmental factors\textsuperscript{8}. In a multicenter Italian study, the APL was diagnosed in approximately 30% of pediatric AML\textsuperscript{9}.

As part of the AML, APL represents a distinct subtype that affects the treatment significantly. The APL is associated with a specific chromosomal abnormality, the t(15;17) translocation. The translocation affects the break point that involves the Retinoic Acid Receptor, producing a "Promyelocytic Leukemia (PML)" protein fusion with "Retinoic Acid Receptor-α (RAR-α)", the rearrangement forms the gene fusion PML/RAR-α, specific marker of disease.

Uncommon APL molecular variants determine characteristic fusion proteins of genes partners (eg, Promyelocyte Leukemia Zinc Finger [PLZF], Nucleophosmin [NPM], Signal Transducer and Activator of Transcription 5-B [STAT5B], and Nuclear Mitotic Apparatus protein [NuMa]) which blend with RAR-α\textsuperscript{10}. APL PLZF/RAR-α variant is characterized by t(11;17) translocation, and represents about 0.8% of APL; it, compared with the classical t(15;17)(q23;q21) APL, expresses CD56 surface antigen and morphologically shows fine intracytoplasmatic granules\textsuperscript{11}.

The rare APL NPM/RAR-α variant shows the t(5;17)(q35;q21) translocation, while the APL NuMa/RAR-α variant shows the t(11;17)(q13;q21) translocation\textsuperscript{12}. The search of these rare variants is important because it affects sensitivity to treatment with all-trans-retinoic-acid (ATRA) or arsenic trioxide.

The PLZ/RAR-α variant is associated with poor prognosis and generally it is unresponsive to treatment with ATRA or arsenic trioxide, in contrast NPM/RAR-α and NuMa/RAR-α variants are responsive to ATRA\textsuperscript{13-14}.

**CASE REPORT**

We report the case of a four-year-old girl who arrived at the emergency room of our hospital for the presence of extensive bruising and petechiae located in the lower limbs and significant bleeding gums. The little patient was in a generally satisfactory condition. The heart rate was 133/bm, blood pressure 100/70 mmHg, O\textsubscript{2} saturation 98%. No hepatosplenomegaly and lymphadenopathy. The full blood count showed severe normochromic anemia (Hb = 69 g/L, Ht = 0.20 L/L; MCHC = 344 g/L), mild microcythemia (MCV = 79 fL) and severe thrombocytopenia (PLT = 16 x 10\textsuperscript{9}/L). There was leukocytosis (WBC = 16.2 x10\textsuperscript{9}/L) and the automatic differential leukocyte count (ADVIA 2120i - Siemens Healthcare \textregistered) showed neutrophilia (Neutrophils = 80%). The Perox cytogram identified a atypical homogeneous cells population located on the right which showed high myeloperoxidase enzyme activity (Figure 1A, arrow). The Baso cytogram showed the presence of several cells with impaired nuclear chromatin (Figure 1B, arrow).

Using the cell counter ADVIA 2120i, the Perox cytogram gave an indication of suspect false neutrophilia and the characteristic appearance corresponds, with rare exceptions, to the leukemic promyelocytes. The ADVIA 2120i Baso cytogram, from the conformation of the mononuclear cluster cell, gave an indication of blast nature of the nuclei.
In this case, given the analytical data, cytograms and qualitative flags, the morphological evaluation of peripheral blood smear is mandatory. The leukocytes differential count obtained with light microscopy showed neutropenia (Neutrophils = 0.81x10^9/L), moreover the blood smear showed several blasts (65%) with small and stubby intracytoplasmatic Auer roads, nucleus often atypical with monocytoid appearance or internuclear bridge (Figure 2, A, B, C, D, E).

Figure 1. Perox and Baso leukocytes cytograms (ADVIA 2120i - Siemens Healthcare®).

Figure 2. A) Blasts with bilobed nucleus and intracytoplasmatic microgranules; B) Blast with bilobed nucleus and Auer road (arrow); C) Blasts with incise nucleus, one blast with Auer road (arrow); D) Blast with intracytoplasmatic microgranules and internuclear bridge; E) Three blasts with nuclear atypia, one with Auer road (arrow).
The strong myeloperoxidase expression in blast cells was confirmed by cytochemical staining (Figure 3).

Figure 3. Blasts myeloperoxidase positive.

The blood chemistry tests showed only the lactic dehydrogenase increased enzyme (LDH = 636 U/L; r. v. 230-460 U/L). The clotting tests showed the slight prolongation of Prothrombin Time (PT = 12.2 s; r. v. = 9.5s), as well as D-dimer (D-d = 1000 ng/mL FEU; r. v. < 550 ng/mL FEU ), while the activated Partial Thromboplastin Time and Fibrinogen were normal.

The presence of myeloperoxidase positive blast population oriented towards an AML, and the distinctive morphology of the blasts give evidence for a APL-M3v.

The morphological diagnosis was confirmed by cytogenetic Fluorescence In Situ Hybridization (FISH) probe on peripheral blood that detected in 60% analyzed interphase nuclei, the presence of the specific t(15;17) translocation (nuc ish (PML, RARα) x2 [40/100]/(PMLx3), (RARαx3), (PML with RARαx2) [60/100] (Figure 4).
DISCUSSION

In children the APL is rare. Morphologically the classic APL is characterized by the presence of blasts promyelocytes hypergranulate > 20% with intracytoplasmatic Auer rods that in several cases, for their high number, form the so-called "faggot cells". In the APL-M3v the blasts show abnormal intracytoplasmatic granules, the nuclei are bilobed or have a monocytoid appearance. Three other morphological subcategories of APL were reported: APL with basophilic morphology, APL with M2-like blasts morphology, and APL with M1-like blasts morphology (early promyelocytic). The new WHO classification includes the APL between "AML with recurrent cytogenetic abnormalities", other cytogenetic abnormalities and specific genes involved in AML are AML1/ETO, inv, and MLL. The APL variants genetically are uncommon and in most cases show the transcript PML/RARα isoform bcr1 and bcr2. The immunophenotype frequently expresses the positivity for CD13 and CD33 antigens but does not express HLA-DR, CD34, CD11b. In some studies the CD56 positivity at diagnosis is associated with poor outcome.

Clinically, in children as with adults, the APL is characterized by a high incidence of leukocytosis (WBC > 10x10⁹/L), as well as the APL-M3v, and it is an important
prognostic factor. Patients with WBC > 5x10^9/L or > 10x10^9/L generally have a poor outcome. Furthermore, patients with leukocytosis at diagnosis can present positivity for FMS-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) (FLT3/ITD); the leukocytosis with FLT3/ITD positivity represent a bad prognostic factor. Morphologically over 25% of pediatric APL appear as microgranular APL variant. About 75% of patients at diagnosis have significant coagulopathy (Disseminated Intravascular Coagulation) with major bleeding events that impact on outcome. Compared with other AML, hepatosplenomegaly and lymphadenopathy in APL are uncommon. With regard the gender, some pediatric therapeutic trials reported a slight increase of APL in females, as well as in children with increased Body Mass Index. Compared to conventional therapeutic protocols in AML, the introduction of ATRA, both in children and adults with APL, has significantly reduced the early mortality due to bleeding. This therapeutic approach should lead to not misunderstand the "Retinoic Acid Syndrome", also known as retinoic acid differentiation syndrome. In about 80% of the pediatric cases the introduction of ATRA has led to the cure of APL. The problem of relapse remains open. Several therapeutic trials have experienced, not only in relapse but also in cases of first diagnosis, arsenic trioxide in combination or as a single agent obtaining effective results. Autologous and allogeneic stem cell transplantation, carried out on patients with relapsed APL treated with ATRA, has given satisfactory results. Before the coming of ATRA therapy, APL was characterized generally by fast evolution in exitus. Understanding the molecular pathogenesis of APL and the subsequent introduction of new therapeutic regimens, an important challenge for the cure of the disease was obtained. Another important step will be to understand the different epidemiology of the disease in pediatric field according to geography.

Finally, the case is reported because in pediatric AML the APL is uncommon and, it represents a true emergency in oncohematology and the timing of diagnosis must be very short. In this the laboratory has an important role in recognizing not only the blasts morphology, but also the cyograms interpretation that, in relation to our blood counter analyzer used, gave a strong indication of APL.

CONFLICT OF INTEREST

No conflict of interest is declared.

REFERENCES