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Case Report Open Access

Acute Leukemia Trilineage Blasts Assignment and Epigenetic Mutations

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Abstract

Mixed Phenotype Acute Leukaemia (MPAL) is a heterogeneous group of rare leukemias in which assigning a single lineage of origin is not possible.

We report a rare case of a MPAL without any clear cytological characterisation. Immuno-phenotyping identifies a blastic population co-expressing myeloid, B-lymphoid and T-lymphoid markers. The diagnosis of MPAL is considered and the patient receives chemotherapy targeting both myeloid and lymphoid components, followed by allogeneic hematopoietic stem cell transplantation. DNA-based techniques analyzing B and T-cell clonality identified the presence of partial rearrangements in immunoglobulin and TCR genes. Mutations in DNMT3A, IDH1/2, Flt3, NPM1 and ASXL1 genes were analysed.

We did not observe any mutations in IDH2, Flt3 and ASXL1 genes. A several mutations were in DNMT3A, IDH1 and NPM1 genes, allowing the monitoring of minimal residual disease.

Keyword

Mixed Phenotype Acute leukaemia; Cytology; Immunophenotypage; Molecular profile; Prognosis

Introduction

Mixed-phenotype acute leukemia (MPAL) is a very rare disease possibly arising from a hemopoietic pluripotent stem cell. The diagnostic criteria were based on the scoring system proposed by the European Group for the Immunological classification of Leukemias (EGIL) that was adopted by the WHO 2001 classification. The WHO definition of MPAL is based on the expression of strictly specific T-lymphoid (cytoplasmic CD3) and myeloid (myeloperoxidase [MPO]) antigens, the latter shown by either flow cytometry (FCM) or cytochemistry and/or clear evidence of monocytic differentiation.

The rarity of MPAL and the lack of uniform diagnostic criteria made it difficult to distinct leukemia's characteristics and the best therapeutic approach.

Here, we report for the first time a Tunisian MPAL case including clinical examination, cytogenetic and epigenetic molecular genes status.

Case Report

We report the case of a 30 years old patient admitted to the hematology department in Tunisia for suspected acute leukemia. He was diagnosed to an alteration of the febrile condition, complicated by acute hypoxic pulmonary Community.

Clinical examination and scan found a tumor syndrome with hepatosplenomegaly and superficial lymphadenopathy (bilateral cervical).

The blood count reveals a moderate pancytopenia with anemia normocytic (Hb=10 g/dL), thrombocytopenia (129 g/l) and leukopenia to (1.3 g/l), with neutropenia.

The cytological examination of blood reveals the presence of 21% of indifferenciated blasts. The hemostasis test was normal and biochemical tests found elevated lactate dehydrogenase (LDH) without lysis syndrome.

Cytological examination of bone marrow revealed a significant blasts (57%) infiltration, very heterogeneous in size and morphology (Figure 1). On one hand, there was a small contingent of blasts with a high Nucleocytoplasmic ratio whose morphology consisted with lymphoblasts. On the other hand, there were large blast cells, with extensive cytoplasm containing sometimes fine granulations. Within these large blasts, some have a morphology monocytoid other megakaryocytic. An erythrophagocytosis was noted in some fields. Overall, the diagnosis of acute leukemia (AL) is proposed, difficult to classify cytologically.

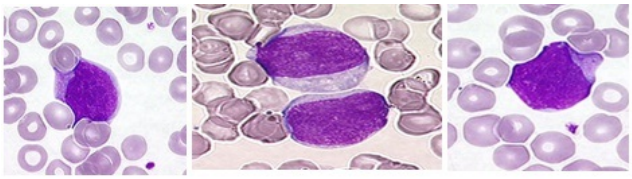

Figure 1: Blasts morphology.

Immunophenotyping of marrow cells showed an expression of CD45 in blasts cells with a high intermediate intensity, and a positivity of immaturity HLA-DR and CD34. Blasts express the specific markers of the myeloid lineage CD33, CD13, CD15, CD65 +, CMPO +, CD11b

+, but are negative for the other monocyte markers (CD14, CD64) (Table 1).

In addition, there is the unexpected expression of CD19 B lymphoid marker, CD22 +, CD10 and lymphoid T marker CD3, CD5+, CD7+ and expressed as intra-cytoplasmic with low intensity.

This immunophenotyping showed a blast coexpressing CD45 with high and intermediate immaturity and trilineages phenotypes.

	Immaturity	Myeloid	B Lymphoid	T Lymphoid
Markers	CD34	CD13	CD19	CD5
	HLADR	CD11b	CD22	CD7
		CD33	CD10	CD3
		CD15	CD79a	
		CD65		
		Cytoplasmic MPO+		

Table 1: Positive antigen expression by blasts cells.

The study of rearrangements of VDJ heavy chain genes (VHDHJH) immunoglobulin (Immunoglobulin heavy chain or IgH) conducted in the University Hospitals Pitié-Salpêtrière Charles Foix, Department of Biological Hematology, and Paris. It highlights a DH-JH rearrangement intensive and two rearrangements of genes TCRδ high intensity. The molecular study concluded to the presence of a clonal population very immature with both partial rearrangements of lines B and T. (Table 1).

Cytogenetic studies of the karyotype and technique fluorescence in situ hybridization (FISH) reveal the presence of mitosis hyperploid in 50% of cells analyzed.

Molecular studies have not demonstrated fusion transcript of BCR-ABL1, rearrangement of the MLL gene or mutation of the FLT3 gene and ASXL1 gene. They highlight mutations in genes; DNMT3A, NPM1 and IDH1.

DNMT3A is a member of a DNA methyltransferase (Mtase) family. Different types of DNMT3A mutations such as missense, frameshift, nonsense splice-site mutations, and deletions were detected, of which missense mutations most commonly are heterozygous, and almost at codon R882 in exon 23 DNMT3A Mtase domain modify its enzymatic activity and has certain gain of function and/or dominant negative effects [1].

DNMT3A mutations are significantly enriched in patients with intermediate risk cytogenetics with a normal karyotype. The frequency of DNMT3A mutations varies from 15% to 40% in AML de novo. Several recent studies have reported an association between DNMT3A mutation and Flt3mut and have a poor clinical outcome in AML patients [2]. In this case we report one single-nucleotide polymorphism L901L, c.2703C>T, and no mutation in Flt3.

IDH, encodes a nicotinamide adenine dinucleotide phosphate, dependent enzyme for oxidative decarboxylation of isocitrate, has an essential role in the tricarboxylic acid cycle and involved in the cellular defence of oxidative damage. Molecular alteration of IDH1 was observed in this AML case with a somatic point mutation R132H, this mutation may contribute to oncogenesis, histone and DNA demethylases, leading to histone and DNA hypermethylation and finally a cell differentiation block [3].

NPM1 is a nucleolar phosphoprotein involved in many cellular processes, Mutant NPM1 is known to bind to and alter the subcellular. We have report in this case an NPM1 mutation in exon 12.

Discussion

This is a case of acute leukemia AL, a young Tunisian man with a clinical presentation very aggressive. Certain immunophenotypic parameters suggest the diagnosis of mixed phenotype acute leukemia. These leukemias are rare represent less than 2-3% of acute leukemias.

The MPALs correspond either to AL with two distinct blast populations and different lineages, or to ALs whose blasts co-express antigens of several lines. Among these MPALs, two particular entities are described: the ALs associated with the translocation t (9; 22) and those with the MLL rearrangement. Other than these 2 subcategories, we speak of MPAL NOS (Not Otherwise Specified)[4].

Therefore, it was decided in a multidisciplinary consultation meeting to take charge of this AL as an acute leukemia of mixed phenotype (T /myeloid). Thus the demonstration of the TCRδ gene rearrangements is of interest for the evaluation of the MRD.

The diagnosis of MPAL is retained and the patient is treated with myeloid and lymphoid chemotherapy for HSC transplantation. The molecular profile retained is NPM1 + / DNMT3A + / IDH1 +.

In DNMT3A gene one single-nucleotide polymorphism L901L that was detected, but did not alter the amino acid residues and have no biologic effect. IDH1R132H mutation was observed which is a mutational hotspot. Somatic point mutation in IDH1, R132 confer a gain-of-function in cancer cells, resulting in the accumulation and secretion of an oncometabolite, the D-2-hydroxyglutarate (D-2HG). Overproduction of D-2HG interferes with cellular metabolism and epigenetic regulation, contribute to oncogenesis. Also, high levels of D-2HG inhibit α-ketoglutarate-dependent dioxygenases, histone and DNA demethylases, leading to histone and DNA hypermethylation and finally a cell differentiation block [5]. Mutant-IDH1 enzyme may represent a novel drug class for targeted therapy. For this patient we cannot confirm that this mutation contributed to the leukemogenisis but NPM1 mutation could a primary genetic lesion, several Recent studies have shown that the *NPM1* mutant perturbs hemopoiesis in

experimental models and that the biologic and clinical features of *NPM1*-mutated AML do not seem to be significantly influenced by concomitant other aberrations or multilineage dysplasia [6]. It associated with a favorable prognosis with the absence of FLT3-ITD and represent a potential MRD marker, and that's the case where we report an NPM1 mutation in exon 12 with a positive minimal residual disease at J42.

The 30 years old patient underwent a bone marrow transplant and responded well.

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