The Role of Immunophenotyping in Diagnosis of Biphenotypic Acute Leukemias

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Introduction: The majority of acute leukemias can be classified as myeloid, B, or T lymphoid. In some cases this is not possible because of the evidence of expression of both lymphoid and myeloid lineage-specific antigens in the blast cells. These cases were defined as biphenotypic acute leukemias. The aim of this study was to present the importance of immunophenotyping in diagnosis of biphenotypic acute leukemias.

Material and method: In our report we present 8 cases of biphenotypic acute leukemia from a total of 218 patient diagnosed with acute leukemia. We performed immunophenptyping of bone marrow samples. Three-or four-color immunoflourescence staining was used. The diagnosis was established according to EGIL (European Group for the Immunological Classification of Leukemias) classification.

Results: Immunophenotyping identified 4 cases with B-lymphoid+myeloid immunophenotype, 2 cases had T-lymphoid+myeloid immunophenotype, 1 patient had B+T-lymphoid immunophenotype and 1 patient had biclonal AL. Except one patient, all of them had blasts positive for CD34 marker. 4 of the patients were treated with acute lymphoblastic leukemia protocol, 2 with acute myeloblastic leukemia protocol and 2 cases with acute myeloblastic leukemia protocol after failure of ALL protocol. The outcome was poor, the median survival was 4 months.

Conclusions: Immunophenotyping of blasts cells is indispensable in the diagnosis of biphenotypic acute leukemia. The most common immunophenotype is co-expression of myeloid and B-lymphoid markers and co-expression of myeloid and B-lymphoid markers. Most cases show expression of stem cell marker CD34. The outcome of this type of leukemias are poor.

Keywords: biphenotypic acute leukemia, immunophenotyping, CD34

Introduction

The diagnosis of acute leukemias (AL) is based on clinical features and paraclinical findings including morphology, immunophenotyping, cytogenetic and molecular investigations. In most of the cases, data obtained from these various sources allow us to assign the malignant cells to a given lineage and differentiation stage. Acute leukemias are classified as having myeloid or B-lymphoid or T-lymphoid lineage based on the expression of surface or cytoplasmic antigens of blast cells, as well as on morphological features.

But there are rare cases of AL in which is very difficult to classify the blasts, they having morphologic, cytochemical and immunophenotypic characteristics of both myeloid and lymphoid lineages. This type of entity was defined as bilineage AL, or biphenotypic AL (BAL) [1]. In bilineage AL there are two separate blast populations, with each population expressing markers of a distinct lineage. In contrast, BAL is characterized by one blast population that co-expresses several myeloid and lymphoid antigens in the same cells.

The criteria for defining acute biphenotypic leukemias was formulated by the European Group for the Immunological Classification of Leukemias (EGIL). They proposed a scoring system to distinguish cases of biphenotypic acute leukemia from classical AL expressing aberrantly one or two markers of another lineage. This system is based on the number and degree of specificity of the markers (lymphoid and myeloid) expressed by the leukemic cells [2]. In 1998, the EGIL redefined this scoring system attributing 1 point for the CD117 (c-kit), after showing strong relationship between this marker and myeloid lineage [3].

The aim of this study was to present the importance of immunophenotyping by flowcytometry in diagnosis of BAL using scoring system proposed by EGIL.

Material and method

Patients

In this study 8 cases of BAL were included from a total of 218 patients diagnosed with acute leukemia in the Clinical Hematology and Bone Marrow Transplantation Unit, Flowcytometry Laboratory between 2003 and 2010. In all of the 8 cases we analyzed the clinical features such as the presence of hepatosplenomegaly, central nervous system (CNS) involvement and laboratory values: white blood cell (WBC)

Table I. EGIL scoring system for biphenotypic acute leukemia [4]

	B-lineage	T-lineage	Myeloid lineage
2 points	CD79a cμ cCD22	CD3 TCR	MPO
1 point	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CD65 CD117
0.5 point	TdT CD24	TdT CD7 CD1a	CD14 CD15 CD64

Table II. Clinical and laboratory features of the patients at diagnosis

No	Age	Sex	WBC ($\times 10^9$/l)	Hb (g/dl)	Plt ($\times 10^9$/l)	% blasts in BM	LDH (U/l)	Hepatospleno-megaly	CNS	Therapy	Survival (months)
1	28	F	15.9	10.0	235	59	1031	+/+	-	ALL/AML	7
2	30	M	59.7	4.7	28	43/39*	412	+/+	+	ALL/AML	6
3	18	M	5.0	10.8	170	84	557	-/-	-	AML	2
4	55	F	2.0	7.8	81	71	825	-/-	-	ALL	2
5	55	F	5.6	11.6	76	85	567	-/+	-	ALL	3
6	19	F	22.0	10.9	125	70	490	+/-	-	ALL	6
7	31	M	22.4	9.6	48	73	510	-/-	-	ALL	4
8	55	M	2.0	15.1	248	75	334	-/-	-	AML	2

Abbreviations: F = female; M = male; ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia, + = present; – = absent; *biclonal AL with 43% myeloblasts and 39% lymphoblasts

count, hemoglobin (Hb) level, platelet count (Plt), serum LDH level. Morphologic examination of peripheral blood (PB) and bone marrow (BM) smear was performed after stain by standard technique with May Grünwald Giemsa.

Immunophenotyping

Whole BM samples were stained using a stain-and-lyse direct three or four color immunofluorescence tehnique. The monoclonal antibodies (MoAb) used for diagnosis of AL were: for B-lineage: CD19, CD22, CD20, CD10, for T-lineage: CD2, CD3, CD5, CD7, CD4, CD8, for myelo-monocytic lineage: CD13, CD33, CD15, CD11b, CD11c, CD16, CD64, CD14, other cells surface markers: CD34, CD117, HLA-DR and intracellular MoAb: CD79a, CD3 and MPO. Sample preparation and flowcytometric data aquisition was performed on day of collection. The BM samples were incubated 20 minutes at room temperature in dark with 5–20 µl MoAb, according to the manufacturers recommandations. After lysing the non-nucleated red cells with 2 ml/tube of FACS Lysing solution (Becton Dickinson Biosciencies – BDB), cells were centrifugated 5 minutes at 540 g in phosphate-buffered saline (PBS) and resuspended in 0.5 ml of PBS/tube. The intracellular staining was performed by fix and permeabilization technique. All MoAb were obtained from BDB. Data aquisition was performed on FACSCalibur (BDB) cytometer using Cell-QUEST software. A total of 50000 events/tube were aquired corresponding to the total nucleated BM cells. The blast gate was identified on side scatter / CD45 dot plot. Data analysis were performed with Paint-a-Gate software. Surface antigen expression was considered positive if at least 20% of blasts showed a positive labeling. For cytoplasmic antigen expression, the threshold was 10%.

Diagnostic criteria

Diagnosis of AML or ALL was performed according to FAB classification and BAL according to EGIL classification. We identify BAL the cases with a score higher than 2 in more than one lineage. In table I we show the EGIL scoring system for BAL [4].

Results

3.6% of cases of acute leukemias diagnosed in our laboratory were identified as BAL (8 cases from a total of 218 patient with AL). The median age of the patients was 36 years, range: 18–55, 4 males and 4 females. Clinical and laboratory features of the patients at time of diagnosis are shown in table II.

Morphologically, in 4 cases the blasts had lymphoblastic morphology, in 3 cases myeloblastic morphology and 1 case had a dual population of small and large blasts difficult to classify by morphology. In table III we show the immunological markers of 8 patients with BAL.

Immunophenotyping identified 4 cases with B-lymphoid+myeloid immunophenotype (IF) (figure 1,2), 2 cases had T-lymphoid+myeloid IF, one patient had B+T-lymphoid IF.

One patient had biclonal AL, one clone with B-lymphoid lineage markers and one clone with myeloid lineage markers (figure 3).

Table III. Immunological markers of 8 patients with biphenotypic acute leukemia

	B-lymphoid markers						T-lymphoid markers						Myeloid markers							
	79a	22	19	20	10	Score	3	2	5	8	7	Score	MPO	13	33	117	14	15	64	Score
1	–	–	–	–	–		+	+	+	–	+	4.5	+	+	+	ND	–	–	–	4
2	+	+	+	+	+	7	–	–	–	–	–		+	+	+	ND	+	–	–	4.5
3	+	+	–	–	–	4	–	–	–	–	–		+	+	+	+	–	+	+	6
4	+	+	+	+	–	6	–	–	–	–			+	+	–	–	–	+	–	3.5
5	+	+	+	–	–	5	+	+	+	–	+	4.5	–	+	–	–	–	–	–	
6	+	+	+	–	–	5	–	–	–	–	–		+	+	–	–	–	+	–	3.5
7	–	–	–	–	–		+	–	+	–	+	3.5	+	+	+	–	–	–	–	4
8	+	+	+	–	–	5	–	–	–	–	–		+	+	+	–	–	–	–	4

Abbreviations: + = present; – = absent; ND = not determined

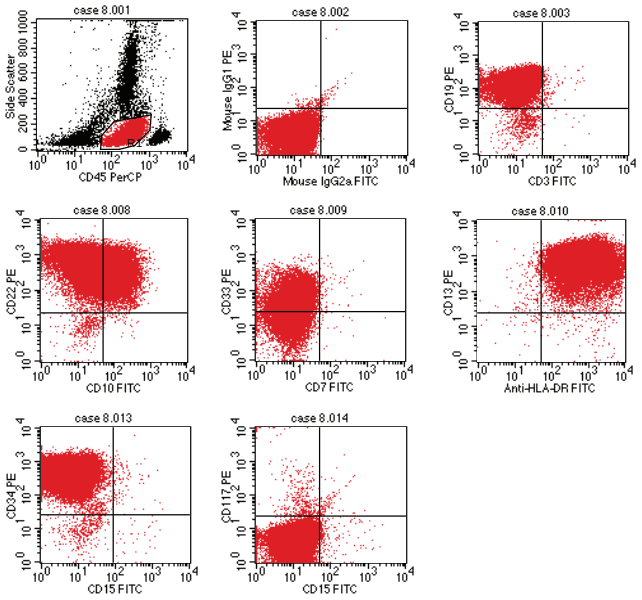

Fig. 1. Case 8: The blasts are positive for B-lymphoid markers: CD19, CD22, CD10, myeloid markers: CD33, CD13 and for CD34, HLA-DR.

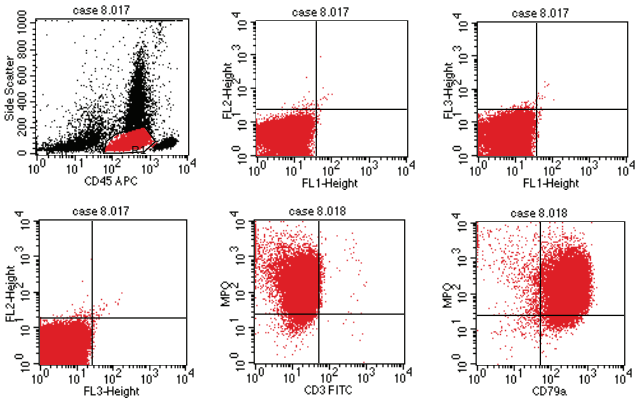

Fig. 2. Case 8. The blasts are positive for intracellular antigens: MPO, CD79a and negative for CD3.

Except one patient, case 8, all of them had blasts positive for CD34 stem cell marker. 4 of the patients were treated with acute lymphoblastic leukemia (ALL) protocol, 2 with acute myeloblastic leukemia (AML) protocol and 2 cases with AML protocol after failure of ALL protocol. The outcome of this patient was poor, median survival was 4 months, range: 2–7 months.

Discussion

Biphenotypic acute leukemia (BAL) is a rare entity that comprises 0.5–4% of all acute leukemias [5], in our laboratory 3.6% of AL were identified as BAL.

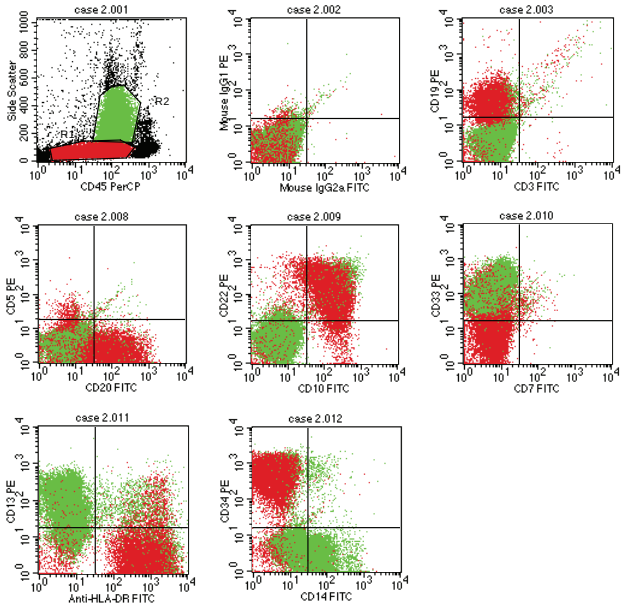

Fig. 3. Case 2. Biclonal AL, one clone with B-lymphoid lineage markers (thin arrow) positive for: CD19, CD20, CD22, CD10 and CD34, HLA-DR and one clone with myeloid lineage markers (thick arrow) positive for CD33, CD13, CD14.

Morphologically the blasts were heterogeneous in our patient similar with other studies [6]. Most of the patients present either as ALL or as AML morphology and in rare cases there is evidence of distinct dual blast population with either lymphoid or myeloid features.

The most frequent immunophenotype was B-lymphoid + myeloid followed by T-lymphoid + myeloid immunophenotype. In one report from Xu the autors compaired 9 other published studies of between 19 and 63 cases of BAL. They find that the most frequent type of biphenotypic AL (BAL) involves the co-expression of markers of myeloid- and B-lineage, between 47 and 72%. BAL with myeloid and T-lineage markers are next in frequency, around 24%, while both B/T and triple myeloid/B/T BAL are rare [5].

The origin of blasts cells in BAL is unknown, it is possible that this leukemia arises in a very early hemopoietic progenitor with potential to undergo either on myeloid or lymphoid differentiation or rarely B- and T-cell differentiation [7]. Most cases of BAL show expression of early hemopoietic stem cell marker CD34 [3,5,7], BAL without CD34 are rare [8]. In our cases the CD34 was positive with the exception one case.

Published studies have documented the poor outcome of BAL [7,9,10]. In our patients the median survival was 4 month. Matutes' et al report suggest that ALL-directed treatment seems more effective with a higher response rate and better outcome compared with an AML or to an AML + ALL schedule. They consider that BAL patients should be candidates for consolidation with intensive chemotherapy and stem cell transplantation at first remission, particularly in those who achieve CR but remain positive for minimal residual disease [6].

Conclusions

Immunophenotyping of blasts cells is indispensable in the diagnosis of BAL.

The most common immunophenotype in BAL is co-expression of myeloid and B-lymphoid markers and co-expression of myeloid and B-lymphoid markers. Most of BAL cases show expression of stem cell marker CD34,

probably arises from multipotent progenitor cells.

The outcome of BAL are poor. The optimal approach for BAL therapy is unknown. Thus, it is important to elucidate the origin of the neoplastic cells for determination of the appropriate therapy.

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