Immunophenotypic Diagnosis of Acute Lymphoblastic Leukemia using Flow Cytometry; Experience at King Hussein Medical Center

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ABSTRACT

Objective: To identify the importance of flow cytometry (FCM) in diagnosis and subclassifying acute lymphoblastic leukemia, and to highlight its capability to detect antigen aberration.

Method: The Results of flow cytometry for 165 patients, between January 2006 and December 2011 who were diagnosed with acute lymphoblastic leukemia (ALL) were retrospectively reviewed with respect to age and gender distribution and immunophenotypic findings.

Results: 63% of patients were children (104 out of 165 patients) with age less than fourteen years old. 114 patients were male while 51 patients were female with male to female ratio 2.2: 1. Precursor-B- acute lymphoblastic leukemia represents eighty percent (132 patients) of cases, of 106 patients (87.6%) were [CD10/CD19] positive,125 patients (94.7%) were positive for cytoplasmic CD79a, 126/129 (97.6%) were positive for HLA-DR, and 15 patients (11.36%) were CD10 negative. Aberrant myeloid antigen expression was noted; CD33 and CD13 were positive in 15/113 (13.3%) and 2/108 (1.85%) respectively. On the other hand precursor-T- acute lymphoblastic leukemias were found in thirty three patients, 84.4% of them were Anti-TdT positive, and all were negative for B-cell markers. Myeloid antigen expression results were as follows; 1/29 (3.4%) and 2/29 (6.9%) positive for CD33 and CD13 respectively

Conclusion: Flow cytometry is a golden tool in diagnosis and identifying ALL subtypes. Precursor-B- acute lymphoblastic leukemia represents most of ALL cases with minority of cases are CD10negative. Aberrant myeloid antigen expression would not change the diagnosis of ALL in either B- or T- subtypes. Further clinical correlation is needed to figure out aberrant markers prognostic implications.

Key words: Acute lymphocytic leukemia, Flow cytometry, Immunophenotype.

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Introduction

Flow cytometry (FCM) is a lab technology that can define leukocyte surface proteins (antigens) using specific antibodies. These antibodies are given so-called CD numbers which stand for cluster of differentiation and refer to the group or cluster of antibodies all of which define a particular protein that differentiates cells of one type. More and more antigens are defined each year, and the CD numbers now range from CD1

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through CD170.[1,2] Antibodies against these antigens have allowed immunologists to define leukocytes that are microscopically indistinguishable from each other but whose concentrations vary in ways that are related to an individual's immune status. For example, B lymphocytes and T lymphocytes look identical under light microscope, but they possess different membrane proteins that allow them to be distinguished by specific antibody staining.[1,2] Some of the membrane antigens on leukocytes define function, some define lineage, and some define developmental stage. Flow cytometry can identify several cellular properties, including size, cytoplasmic and nuclear complexity. It can provide correlated data that links different population profiles all based on single cell analysis.[1,2] Flow cytometry is important diagnostic and prognostic modality for different hematologic malignancies including leukemias. Moreover, It can also be used to monitor the effectiveness of treatments and to detect minimal residual disease.[3-9] In acute lymphoblastic leukemia, (FCM) can identify the lineage and stage of maturation using a panel of antibodies.[5,10] The main goal of this study is to emphasize the importance of FCM as a tool for diagnosis andsubclassifying ALL cases and to demonstrate the frequency of different CD markers in both T- and B ALL patients at Kinfg Hussein Medical Center (KHMC).

Method

The flow cytometry results of one hundred and sixty five samples of bone marrow (BM) and/or peripheral blood (PB), diagnosed as de novo ALL were reviewed retrospectively. The initial FCM reports were obtained at time of diagnosis over a six year period between January 1st 2006 and December 31st 2011at King Hussein medical center (KHMC) in Jordan. The demographic information including age, gender was all noted. Acute leukemia diagnosis was established using conventional tools (PB and BM studies) using 20% blasts as a cut-off point for diagnosis. Bone marrow aspirate smears and peripheral blood specimens were obtained and processed using standard protocol by using Wright-Giemsa technique and visualized under light microscopy. The bone marrow biopsy specimens were fixed, stained, and processed using routine protocols for most cases.[5] Flow cytometry analysis using two-color instrument was performed using standard protocols.[6]

Results

A total of 165 cases were reviewed; 51 females and 114 males with a male to female ratio (M: F) of 2.2:1. The cases were divided according to gender and age group as shown by table 1. Of the total, 132 patients (80%) were of pre-B-ALL types, the M: F was 1.87:1 and most cases were within the pediatric-age group (2-15 years). Pre-T-ALL represented the minority of ALL cases with striking male predominance as M: F was 5.6:1, similar to pre-B-ALL; most cases were within the pediatric-age group (Table I). The immunophenotypic pattern for pre-B-ALL cases (Table II) was as follows; 106 patients (87.6%) were [CD10/CD19] positive, 22 patients (16.6%) were CD19 positive, 125 patients represent (94.7%) were positive for cytCD79a, 126/129 (97.6%) were positive for HLA-DR, and 15 patients (11.36%) were CD10 negative. Aberrant myeloid antigens expression was noted; CD33 was positive in 15/113 (13.3%), whereas CD13 was lower with 2/108 (1.85%). The CD10 negative cases results were displayed on Table III, two third of which are adults with equal M: F. All cases in this group were negative for myeloid and T-cell markers. The flow cytometry results for pre-T-ALL shown in Table IV were as follows: all cases were positive for cytCD3 and all were negative for B-cell markers (CD19, CD20, CD22 and cytCD79a). Myeloid markers results were as follows; 1/29 (3.4%) and 2/29 (6.9%) positive for CD33 and CD13 respectively. TdT marker positivity was traced in all cases, pre-B-ALL showed the higher percentage of positive cases of 98% (Fig. 1).

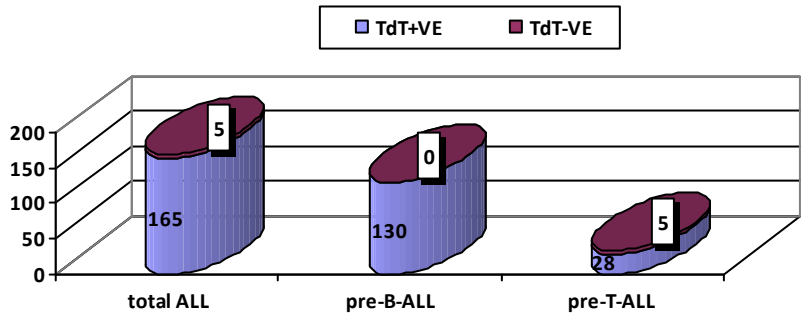

Fig. 1: TdT in each ALL subgroup

Table I: Age and gender distribution of ALL patients

	Pre-B-ALL patients	Pre-T-ALL patients	Total ALL patients
Number	132 pts (80%)	33 patients (20%)	165 patients
Female	46 pts (34.9%)	5patients (15.15%)	51patients (30.9%)
Male	86 pts (65.1%)	28patients (84.85%)	114patients (69.1%)
M:F	86:46 1.87:1	28:5 5.6:1	2.2:1
Age-Pediatric (2-15yr)	83 pts (62.9%)	21patients (63.6%)	104patients (63%)
Age-Adult (16-65 yr)	49 pts (37.1%)	12patients (36.4%)	61 patients (37%)

Table II: Pre-B-ALL Flow cytometry results

Pre-B-ALL Flow cytometru results			
Marker	**Satus**	**Result**	
CD10	negative	15	12.4%
CD2	negative	107/109	98.1%
(Cyto-CD3) (CD3)	negative	120/121	99.1%
CD4	negative	50/50	100%
CD5	negative	22/22	100%
CD7	negative	100/100	100%
CD8	negative	58/58	100%
CD10/CD19	positive	106/121	87.6%
CD22	positive	26/27	96%
CD Cyt-79a	positive	125/125	100%
TdT	positive	130/132	98%
HLA-DR	positive	126/129	97.6%
MPO	negative	91/91	100%
CD117	negative	39/40	97.5%
CD13	negative	106/108	98.1%
CD33	negative	98/113	86.7%
CD33	positive	15/113	13.3%
CD13	positive	2/108	1.85%
CD34	positive	22/32	68.75%

#/#: Positivity for designated marker/number of cases have been done for the corresponding marker

Table III: flow cytometryfindings in CD10 (-ve) pre-B-ALL

Pre-B-ALL with CD10(-ve)	
CD 2 – negative	15/15
CytCD3 – negative	13/13
CD4 – negative	6/6
CD5-negative	4/6
CD7 - negative	9/9
CD8 - negative	5/5
CD13- negative	9/9
MPO negative	12/12
CD19- positive	13/13
CytCD79a- positive	15/15
TdT- positive	15/15
HLA-DR- positive	14/15
CD 34- positive	10/11
2/3 are adult	male Equal female

Table IV: Pre-T-ALL Flow cytometry results

Pre-T-ALL Flow cytometry results			
Marker	**Status**	**Result of done patients**	
CD1a	Positive	18/18	68.4%
CD2	Positive	24/25	96%
CD3	Positive	7/8	87.5%
Cyto-CD3	Positive	30/30	100%
CD4	Positive	14/23	60.87%
CD5	Positive	13	<40%
CD7	Positive	25/26	96.1%
CD8	Positive	2/18	38.9%
TdT	Positive	27/32	84.4%
CD10	Negative	28/32	87.5%
CD13	Negative	27/29	93.1%
CD19	Negative	27/27	100%
CD20	Negative	21/21	100%
CD22	Negative	24/24	100%
CD20 or CD22	Negative	29/29	100%
Cyt CD79a	Negative	32/32	100%
HLA DR	Negative	27/30	90%
MPO	Negative	15/15	100%
CD117	Negative	10/11	90.1%
CD33	Negative	28/29	96.5%
CD33	Positive	1/29	3.4%
CD13	Positive	2/29	6.9%
CD34	Positive	21/31	67.7%

Discussion

CD marker detection is a powerful tool in term of discriminating different hematopoietic cells. Multicolor FCM provides the opportunity to evaluate multiple antigens simultaneously, making it possible to characterize various cell populations in a more precise manner.[1,2,8,9] Moreover, help in differentiation acute myeloid leukemia (AML) from ALL and its subtypes, as well as revealing mixed-lineage leukemias subtypes.[1,2,10-12] There are three major subtypes of precursor B-cell ALL as follow: 1st subtype: pro-B-ALL with CD10 negativity, and no surface or cytoplasmic immunoglobulin (Ig). The 2nd subtype: Precursor B-cell ALL CD10 positive, and no surface or cytoplasmic immunoglobulin Ig. Approximately three-quarters of patients with pre-B-cell ALL have the common precursor B-cell immunophenotype and have the best prognosis. Other pre-B-cell ALL presence of cytoplasmic Ig, and shows in 25% of patients t(1;18) translocaton[13,14] The 3rd subtype approximately 2% of patients present with mature B-cell leukemia (surface Ig expression, generally with French American British (FAB) L3 morphology and a translocation involving the C-MYC gene.[12]

A distinct subtype of childhood T-cell ALL, termed early precursor T-cell ALL, which was identified by flow cytometry.[9] It is characterized by a distinctive immunophenotype of CD1a and CD8 negativity, with weak expression of stem cell or myeloid markers and weak expression of CD5.[11] A retrospective analysis suggested that this subset may have a poorer prognosis than other cases of T-cell ALL.[12] According to WHO classification, the presence of myeloperoxidase (MPO) is required to establish the myeloid lineage. An ambiguous lineage represents less than 5% of acute leukemia in children, expressing features of myeloid and lymphoid lineage [13–15]. Expression of aberrant myeloid antigens reportedly occurs in 5–22% of pediatric patients with de novo ALL.[15] Our study showed pre-B-ALL co-expressing myeloid markers CD13 and CD33 of 15/165 patients (9.1%) of total ALL cases, and 15/132 (11.3%) respectively. Precursor B-cell ALL, defined by the expression of cytoplasmic CD79a, CD19, HLA-DR, and other B cell-associated antigens, accounts for 80% to 85% of childhood ALL. Approximately 90% of precursor B-cell ALL cases expresses the CD10 surface antigen. We found 15/132 patients (11.36%) pre-B-ALL CD10 negative in our study, which still within the wide international range 5% to 20%.[8-10] Consolini and et al have found similar results out (95.6%) of patients with B-lineage ALL

expressing CD10 and 46 of 254 (18.1%) with T-cell ALL.[16] A large study has been performed in Japan by Pediatric Leukemia/Lymphoma Study Group[7] Shows that B-lineage and T- ALL responsible for 87 and 13 % of childhood ALL patients, respectively. More than 90% of early pre-B ALL cases pressed CD19, CD79a, CD22, CD10 and TdT. Cytoplasmic CD3 and CD7 antigens were positive in all T-ALL specimens. More than 80% of T-ALL cases expressed CD2, CD5 and TdT. We showed almost similar results for both B- and T- ALL except for CD5 where less than 40% of our T-ALL cases showed positive expression. Further study to include more sample number and to figure out clinicopathological correlation is needed in our patients to find out this deviation. Regarding expression of CD13 and CD33 was significantly more frequently associated with B- ALL than with T-ALL which supports our results.

Recommendations

A Consensus acute leukemia and ALL panels should be adopted as a routine standard of care in our lab. Well-defined guidelines and algorithm should be implemented and standardized throughout all hematology laboratories worldwide.

Limitations of the Study

We did not look into the survival rate and its relation to various immunophenotypic findings, as well as the use of two color flow cytometry,both represent limitations in our study.

Conclusion

Flow cytometry is a golden tool in diagnosis and identifying ALL subtypes. Precursor-B-acute lymphoblastic leukemia represents most of ALL cases with minority of cases with CD10negative. Myeloid antigen expression would not change the diagnosis of ALL in either B- or T- subtypes. Further clinical correlation is needed to figure out aberrant markers prognostic implications.

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