Immunophenotypic Profile of Acute Leukemia Cases Using Multicolor Flow Cytometry; Three Year Experience at King Hussein Medical Center

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ABSTRACT

Objective: To study the immunophenotypic profile of acute leukemia cases, using multicolor flow cytometry for lineage subtyping.

Methods: This is a retrospective review of acute leukemia cases conducted at department of Hematopathology at King Hussein Medical Center between January2011 to December 2013. A total of 340 acute leukemia cases were analyzed using flow cytometry method. The diagnosis was based on morphological assessment of peripheral blood and bone marrow aspirate smears and immunophenotyping by flow cytometry.

Results: A total of 340 cases of acute leukemia were studied. 164 cases (48.2%) were acute lymphoblastic leukemia, 176 (51.8%) were acute myeloid leukemia. Acute leukemia was diagnosed among adults in 51.8% whereas 48.2% were children. Of the acute lymphoblastic leukemia cases, 130 cases (79.3%) were B-cell type and 34 cases (20.7%) were T-cell type. All cases of B-acute lymphoblastic leukemia showed expression of pan B-cell markers (CD19,CD22 and cytoplasmic CD79a) and 117 (90%) of cases expressed CD10. Cytoplasmic CD3 and CD5 were the most sensitive markers for diagnosis of T-acute lymphoblastic leukemia. Of the 176 cases of acute myeloid leukemia, 16 cases (9%) were identified as acute promyelocytic leukemia, while the rest 160 cases showed expression of CD34 and HLA-DR in 41.4% and 68.7% retrospectively. None of the cases of acute promyelocytic leukemia were positive for both CD34 and HLA-DR. CD13 and CD33 were expressed in all cases of acute myeloid leukemia studied.

Conclusion: Flow cytometric immunophenotyping is a powerful method for accurate diagnosis, identification and subtyping of acute leukemia. Furthermore, it has a great therapeutic and prognostic implications on such cases with unique usefulness in differentiation between acute lymphoblastic leukemia and acute myeloid leukemia-M0. Immunophenotyping results of acute leukemia in this group of Jordanian patients were comparable to the international data. By combining morphology and immunophenotyping, we were able to diagnose and classify cases of acute leukemia at our center where peripheral blood and adequate bone marrow aspirates are available.

Key words: Flow cytometry, Leukemia, Immunophenotyping

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Introduction

Acute leukemias are a heterogenous group of hematological malignancy characterized by uncontrolled clonal proliferation arising in hematopoietic progenitor cells with varying clinical, morphologic, immunologic and molecular features.[1,2] Usually replacing the bone marrow, circulating in peripheral blood and involving others (liver, spleen and lymph nodes).[3] The leukemic cells express characteristic antigens either surface or nuclear which can be identified and subsequently facilitate the diagnosis, accurate classification, subtyping and treatment programs.[4]

In the past, the diagnostic accuracy of acute leukemia by morphology and cytochemistry reached about 80%. By using flow cytometry and it's application in the diagnosis of acute leukemia, the ability of proper classification approaches 98%.[5]

This retrospective review, which was conducted at King Hussein Medical Center to study the immunophenotypic profile of various acute leukemias using multicolor flow cytometry and to assess the usefulness of flow cytometry in establishing the diagnosis and subtyping of these cases.

Methods

This retrospective study was conducted at King Hussein Medical Center over a period of 3 years (January 2011- December 2013). A search of our hematopathology department data-base, revealed 340 cases of acute leukemia that were diagnosed and analyzed using flow cytometry method. Clinical and laboratory records of these patients were reviewed. Data including patient's age, gender, types of acute leukemia and immunophenotypic results were collected.

Acute leukemia was diagnosed and classified on the basis of the morphological criteria of peripheral blood and adequate bone marrow aspirate smears and immunophenotyping by 4-color flow cytometry. No cases with dry tap were included in the study.

Regarding morphological and immunophenotypic assessment, all specimens were processed using standard methods. Peripheral blood and bone marrow aspirates were stained with May-Giemsa stain and examined under light microscopy. Samples of peripheral blood and bone marrow were immediately transported in ethylene diamine tetraacetic acid (EDTA) to the flow cytometry laboratory. All samples were processed within 24- hours. According to the standard immunophenotypic analysis, monoclonal antibodies were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine (PC) and then directed to the following antigens: CD10, CD19, cytoplasmic CD22, cytoplasmic CD79a, CD2, cytoplasmic CD3, CD4, CD5, cytoplasmic CD7, CD8, CD34, CD33, CD13, CD117, CD14, CD64, CD11c, Myeloperoxidase (MPO), Human leukocyte antigen-DR (HLA-DR) and non-specific lineage pan-leukocyte (CD45).The typical combinations of antibodies included CD34-FITC/CD117-PE, CD34-FITC/CD19-PE, CD19-PE/CD10-APC, CD7-FITC/CD33PE, CD14-APC/CD34-FITC, CD19-PE/CD20-FITC, CD64-PE/CD4, CD58-PE/CD38-APC, HLA-DR-FITC/CD117-PC, HLA-DR-FITC/CD22-PC, CD15-FITC/CD11b-PE, CD15-FITC/CD11c-APC, CD33-FITC/CD4-APC, CD4-APC/CD8-PE, cCD3-FITC/MPO-PE, cCD79a-FITC/MPO-PE, nuclear Tdt-PE.

For membrane labeling, the samples were incubated with each antibody for 10 minutes then with 2 ml of lysing solution to lyse the erythrocytes. After that the samples were centrifugated for 5 minutes then washed with 2 ml of phosphate-buffered saline. For cytoplasmic and nuclear labeling, a permeabilizing buffer was used. Multicolor flow cytometry was performed on FACScan. The appropriate blast gate was selected using the combination of side scatter (SSC) angle and forward scatter (FSC) angle. To gate on the blast population, CD45 versus SSC as shown in Fig.1 was used and 10.000 events were collected. The cutoff limit for a positive antigen expression was $\geq 20\%$ for all antibodies.[4,5]
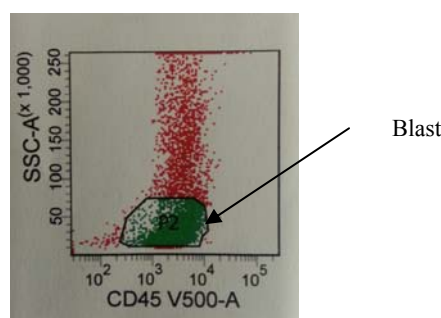

Fig.1: Gating on blast population

Table I: Frequency of each type of acute leukemia according to age groups

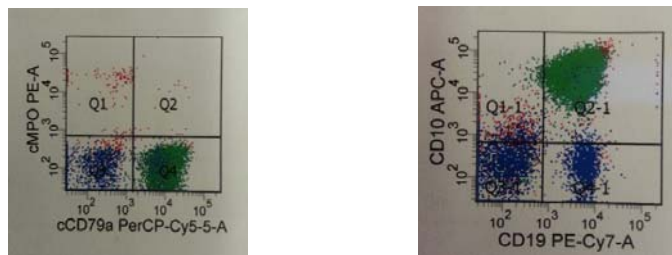| Type of acute leukemia	Children N (%)	Adults N (%)	Total N (%)
ALL	118 (72%)	46 (28%)	164 (48.2%)
AML	36 (20.5%)	140 (79.5%)	176 (51.8%)

Fig. 2: CD 10 positive precursor B-cell ALL
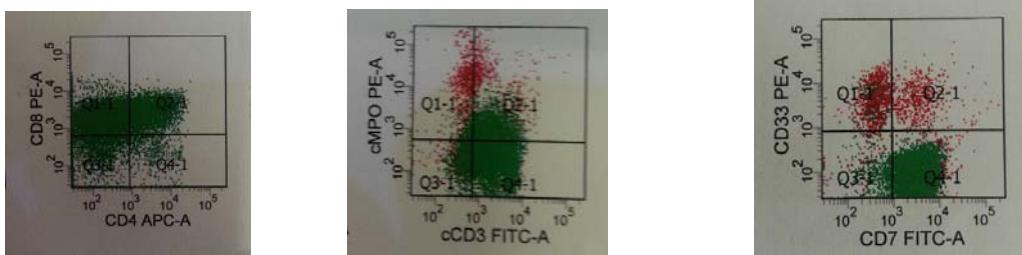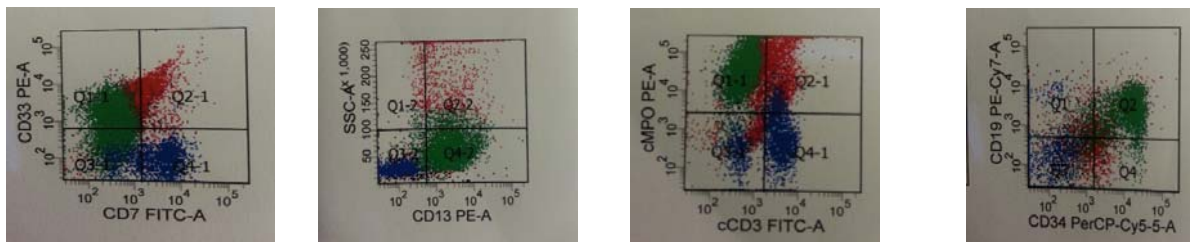

Fig. 3: T-ALL

Fig. 4: AML –M2 with co-expression of CD19

Results

Out of total 340 cases of acute leukemia, based on morphology and immunophenotyping by 4-color flow cytometry, there were 164 cases (48.2%) of acute lymphoblastic leukemia (ALL) and 176 (51.8%) of acute myeloid leukemia (AML). Of all 164 cases of ALL, 130 cases (79.3%) were identified as B-ALL and 34 cases (20.7%) as T-ALL.

The study results showed that 212 (62.4%) were males and 128 (37.6%) were females with male: female ratio of 1.65:1. There were slightly more adults (age greater than 15 years) affected by acute leukemia constituting 176 cases (51.8%) whereas there were 164 cases (48.2%) children with age less than or equal 15 years. The percentage of ALL among children and adults was 72% and 28% respectively while for AML in children and adults 20.5% and 79.5% respectively as shown in Table I. We found that all cases of B-ALL showed expression of pan B-cell marker (CD19, CD22 and cytoplasmic CD79a) and 117 cases (90%) of B-cell lineage ALL express CD10 and classified as CD10 positive precursor B-cell ALL as shown in Fig 2. 13 cases (10%) were CD10 negative. Aberrant expression of myeloid markers CD13 and CD33 was seen in 9 cases (7%) of B-ALL respectively. Regarding T-ALL, cytoplasmic CD3 and CD5 were seen in almost all cases of T-ALL as shown in Fig. 3.

Of the 176 cases with AML, 16 cases (9%) were identified as acute promyelocytic leukemia (APL) based on the morphology and FISH test for the presence of PML/RARA. According to morphology and FAB criteria, the other 160 cases of AML were classified as the following: 8 cases (4.5%) M0, 40 cases (22.7%) M1, 43cases (24.4%) M2 as shown in Fig. 4, 44 cases (25%) M4, 19 cases (11%) M5, 6 cases (3.4%) M6. No

cases of M7 diagnosed. The expression of CD 34 and HLA-DR was seen in 73 cases (41.4%) and 121 cases (68.7%) respectively among various subtypes of AML.

In our study, the brightest positivist of CD34 was seen in M1 and M2 subtypes. None of the cases of the APL were positive for both CD34 and HLA-DR. Two out of 8 cases of AML-M0 express dim (Terminal deoxynucleotidyltransferase) TdT. Aberrant expression of lymphoid markers CD7 and CD19 was seen in 5 cases (2.8%) and 1 case (0.5%) of AML cases respectively.

Discussion

Apart from morphological and cytogenetic methods, immunophenotyping has become the preferred tool for diagnosis, lineage assignment and classification of acute leukemias that lead to the best management programs and for disease monitoring of cases.[4-6]

Acute leukemias account for 352,000 new cases and 265,000 deaths worldwide in 2012.[7] According to the American Cancer Institute, ALL is the most common cancer in children with an annual rate of 35-40 cases per 1 million people in the United States. With peak age of incidence at 2-3years which is approximately fourfold greater than that for older children and adults whereas AML is more common in adults.[8-10] This is in agreement with our study results which demonstrated that the percentage of ALL among children was 72% whereas the percentage of AML in adults was 79.5%. Our study showed that 79.3% of ALL cases as B-cell lineage whereas 20.7% as T- ALL. This is supported by a study from BP Koirala Memorial Cancer Hospital in Nepal during 2- year period (2010 – 2012), which reported that out of total 52 new cases of acute leukemia, 64.5% were B-ALL and 35.5% labelled as T-ALL.[5]

By definition, the diagnosis of B-ALL is assumed if B- cell markers (CD19, cytoplasmic CD79a, cytoplasmicCD22) are expressed in combination or at high intensity, while none of these markers by itself is specific for B-ALL.[11] Furthermore, the diagnosis of ALL of T-cell lineage is based on the presence of cytoplasmic CD3 in all blasts with coexpression of CD5. In T-ALL, there is variable expression of CD1a, CD2, CD3, CD4, CD5, CD7, CD8.[5,11] In the present study, CD19, CD22 and cytoplasmic CD79a were expressed in virtually all cases of B-cell ALL. Regarding T-ALL, cytoplasmic CD3 and CD5 were seen in almost all cases of T-ALL. This is similar to a recent study done in Mansoura in Egypt by Dalia A. Salem et al. during 2 year period (2009-2010) about the flow cytometric/ immunophenotypic profile of 164 patients with acute leukemia which addressed that cytoplasmic CD79a and CD19 were the most sensitive marker for B-ALL while cytoplasmic CD3 and CD5 were the most sensitive markers for T-ALL.[12]

CD10 (common acute lymphoblastic leukemia antigen CALLA) is expressed by early B, pro-B and pre-B lymphocytes.[13] In childhood B-ALL, CD10 expression has been associated with favourable prognosis as compared to the CD10 negative B-ALL. In adult ALL, the patients have poor prognosis with worse survival rate than children.[5,14] Jmili NB, et al. from Paris studied the antigenic profile of blasts in ALL by flow cytometry in 2009 and found that 80% of B-ALL cases were CD10 positive and it is a marker of better prognosis.[15]

In our study we have showed that 90% of B-cell lineage ALL express CD10 with only 10% labelled as CD10 negative precursor B-cell ALL.

The lymphoblasts in ALL usually expressTdT. TdT expression can be seen in AML with minimal differentiation (M0).[11]With respect to myeloid antigens expression in lymphoid leukemias, the most frequent was CD13, CD33.These markers have been associated with poor prognosis and poor response to chemotherapy targeting ALL.[16]Our study showed that the main myeloid antigen expression were CD13 and CD33. This was similar to the results of Zhang YD et al. from China who found that 23% of ALL cases showed myeloid antigen expression and the most common were CD13, CD33, CD14 and MPO.[17]

Regarding the various subtypes of AML, proper diagnosis of APL is important since it constitutes a hematological emergency that requires specific therapy.[18] Beside classical morphology and typical flow cytometry results (positivity for CD13, CD33, CD117 and negativity for CD34 and HLA-DR), the diagnosis should be immediately confirmed by means of FISH for detection of PML/RARA isoform.[5,18] As shown by our study and most studies, none of these

cases express CD34 neither HLA-DR.

In AML-M0, TdT is expressed in approximately 50% of cases. The immunophenotypic analysis plays an important role in distinguishing between ALL and AML-M0 by expression of myeloid antigens (CD13, CD33, CD117) and lack of lymphoid antigens.[5,11] In our study, about 25% of AML-MO cases express TdT. Patients with TdT positive AML-M0 tend to have a higher peripheral blood and bone marrow blast counts and better overall survival after stem cell transplant compared to patients with TdT negative AML-M0.[19]

The immunologic markers characteristic for monocytic differentiation are CD14, CD4, CD11b, CD64, CD36.[5,11] With respect to lymphoid antigens expression in AML, the most frequent is CD7. CD19 also can be expressed in certain cases of AML with t(8;21).[11] Our study has shown that CD13, CD33 and CD117 are highly expressed in various subtypes of AML. Furthermore, CD14 was expressed only in M4 and M5 and the overall expression rate of CD34 was 41.4% with highest positivity seen in M1 and M2 subtypes. The most commonly expressed lymphoid marker in our study was CD7.This was similar to the results of two studies in China in which, Tong H *et al*. and Yang LL *et al*. analyzed the immunophenotypic features of AML and found that the most common antigens expressed in AML were (CD13, CD33, MPO and CD117), the overall positive rate of CD34 was 57.8% and the most common expressed lymphoid marker was CD7.[20,21]

Limitations of the study

We did not study the cytogenetics, molecular findings of acute leukemia cases or the role of immunohistochemistry on the bone marrow biopsies.

Conclusion

Flowcytometric immunophenotyping is a powerful method in accurate diagnosis, identification and subtyping of acute leukemias. Furthermore, it has great therapeutic and prognostic implications on such cases with unique usefulness in differentiation between ALL and AML-M0. Immunophenotyping results of acute leukemia in this group of Jordanian patients were comparable to the international data. By combining morphology and immunophenotyping, we were able to diagnose and classify cases of acute leukemia at our center where peripheral blood and adequate bone marrow aspirates are available.

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