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# Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia

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**ABSTRACT**

***Purpose:*** The aim of this study was to evaluate the incidence of aberrant phenotypes and possible prognostic value in peripheral and bone marrow blood mononuclear cells of Iranian patients with AML.
***Methods:*** 56 cases of de novo AML (2010-2012) diagnosed by using an acute panel of monoclonal antibodies by multiparametric flowcytometry. Immunophenotyping was done on fresh bone marrow aspirate and/or peripheral blood samples using the acute panel of MoAbs is stained with Phycoerythrin (PE) /fluorescein isothiocyanate (FITC), Allophycocyanin (APC) and Peridinin-chlorophyll protein complex (perCP). We investigated Co-expression of lymphoid-associated markers CD2, CD3, CD7, CD 10, CD19, CD20 and CD22 in myeloblasts.
***Results:*** Out of the 56 cases, 32 (57.1%) showed AP. CD7 was positive in 72.7% of cases in M1 and 28.5% in M2 but M3 and M4 cases lacked this marker. We detected CD2 in 58.35 of M1cases, 21.40% of M2 cases, 33.3 of M3 and 20% of M5; but M4 patients lacked this marker. The CBC analysis demonstrated a wide range of haemoglobin concentration, Platelet and WBC count which varied from normal to anaemia, thrombocytopenia to thrombocytosis and leukopenia to hyper leukocytosis.
***Conclusions:*** Our findings showed that CD7 and CD2 were the most common aberrant marker in Iranian patients with AML. However, we are not find any significant correlation between aberrant phenotype changing and MRD in our population. Taken together, this findings help to provide new insights in to the investigation of other aberrant phenotypes that may play roles in diagnosis and therapeutic of AML.

## Introduction

Leukaemias comprise approximately 8% of the entire human cancers, and around half of these cases are classified as acute leukemia. Acute myelogenous leukemia (AML) increase the proliferation in megakaryocytic, monocytic, granulocytic, and erythrocytic lineages.[1] AML is a heterogeneous disease, which shows a high variety of phenotypes. Immunophenotyping is crucial for diagnosis and for definition of particular AML subtypes.[2] Acute leukemia involves both children (ALL) and adults (AML) with a prevalence rate of approximately 4 million people per year in the developed countries.[1] Morphology and Immunohistochemistry are not such reliable method for leukemia diagnosis and classification; hence, Immunophenotyping become a widely used method to diagnose and classify acute leukemias, first in distinction of acute leukemia from other neoplastic diseases and reactive disorders, second in distinction of AML and ALL and third, in classification of AML and ALL into their subsets.[3] More than 95% of acute myelogenous leukemia cases (AML) cases can be easily distinguished from ALL by surface antigen analysis.

Immunophenotype as well as a variety of clinical and biological parameters examined for potential importance in predicting treatment response and patient's survival. [4] When the morphology interpretatin is difficult, immunotyping can be extremely helpful. The main benefit of using surface antigens by flowcytometry is the identification of particular leukemia subtype that cannot be recognized by morphological criteria. Although Immunotyping of

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peripheral blood (PB) and bone marrow (BM) is not decision making for an exact therapeutic procedure by itself, is a practical prognostic indicator.[5] The diagnosis and management of acute leukemia mainly depend on the detection, identification and characterization of leukemic cells.[6] Some studies show a strong relation between particular immunophenotypes and genetic recurrent.[7] Although many acute leukaemias can be correctly identified morphologically or by immunohistocytochemical analysis, immunophenotyping is still essential for accurate identification of some subtypes of AML such as $M_0$, $M_1$ and $M_7$.

Aberrant phenotype is a phenomenon in which lymphoid-associated and other myeloid lineage markers expressed in myeloblasts or myeloid-associated markers expressed in lymphoblasts. Aberrant phenotype incidence has been reported in both ALL and AML with varying frequencies as high as 88%.[8,9] Up to 48% of AML cases were reported to have aberrant expression of a single antigen associated with lymphoid cell lineage.[8-11] The incidence of the aberrant phenotypes in AML is still controversial and different results have been found by different groups, probably because of the several reasons including, use of a large variety of monoclonal antibody (MoAbs) panels, use of different reagents against the CD surface antigens, different cut-off levels, analysis of fresh or frozen cell material, and most importantly the difference between phenotypic characteristics of blast cells of children and adults patient.[12,13] Currently, Flowcytometeric analysis of leukemic blasts has become significant also for classification of biphenotypic and bilineal acute leukemias too. Recently, although the prognostic value of individual antigen expressions on leukemic cells is still controversial, detection of minimal residual disease by flowcytometery, especially in AML, and monitoring the acute leukemia patients who go into remission become exceedingly important.[14]

Several investigators use dual/multi-color flowcytometric immunophenotyping to identify aberrant phenotype. This allows simultaneous analysis of two light scatter and up to four fluorescence signals. Multiparameter high-resolution flow cytometry has been developed to precisely identify lineage characteristics of leukemia.[6,7]

The aim of this study is to evaluate the incidence of aberrant phenotypes and possible prognostic value in peripheral and bone marrow blood mononuclear cells of Iranian patients with AML.

## Materials and Methods

### *Patient selection*

56 new cases of adult AML, who admitted and diagnosed from 2010 to 2012 in Shahid Ghazi hospital (Tabriz, Iran). AML confirmed by bone marrow aspiration sample and peripheral blood smear (PBS) based on the morphology and immune phenotyping. All cases were characterized according to the French – American –British (FAB) cooperative group based on morphological criteria and classified as follows: M0 (3), M1 (12), M2 (16), M3 (11), M4 (8), M5 (5), M7 (1).

### *Immunophenothyping*

#### *Bone Marrow Samples*

Bone marrow Samples were aspirated from the iliac crest. The specimens were collected by expert oncologist and drawn into EDTA tube.

#### *Peripheral blood samples*

The patients WBC count was adjusted at less than 10x $10^3$/ml. Each sample had more than mentioned count was diluted in order to reach the appropriate count. A specimen which contained any clout is excluded. A peripheral blood smear was prepared for microscopic assessment.

#### *Bone marrow analysis*

A bone marrow smear was prepared for microscopic examination. Bone marrow aspirate was checked for clot existence, if any was present, it was disintegrated with wooden sticks. In order to remove any fat or proteins, the bone marrow sample was washed before the procedure. Washing instruction steps are: adding about 4-5 ml of 2% PBS to 1.5 -2 ml of bone marrow aspirate in test tube, 10 minutes centrifuge at 2500 rpm (sigma. USA), removing the supernatant and resuspending pellet with adequate volume of 2%PBS. These steps repeated two times. After washing process done, cell counting was performed by CBC Analyzer or hemacytometer.

#### *Flow Cytometric Analysis of Acute Leukemia Cases*

*Lysing and Staining:* Ten microliters of fluoresceine isothiocyanate (FITC) conjugated monoclonal antibody, 10µl of Phycoerythrin (PE) conjugated monoclonal antibody, 5µl of Allophycocyanin (APC) conjugated monoclonal antibody, and 5µl of Peridinin-chlorophyll protein complex (perCP) conjugated monoclonal antibody was added to 12 *75-mm tubes, afterward 100µL of whole blood was in each tube. The mixture was Vortexed tenderly and incubated about 45 minutes to 1 hour in the dark area at room temperature (20- 25˚C). Tow ml of 1X FACS lysing buffer was added to incubated mixture. Then it was vortexed tenderly and incubated for 20 minutes in the dark area at room temperature again; after that Centrifuge at 500g for 5 minutes was done. The supernatant was removed. Subsequently 2-3 ml of washing buffer was added and centrifuged at 500g for 5 minutes and the supernatant was removed. 1 ml of 1% cell fix (paraformaldehyde solution) was added and mixed completely, analysis can be done immediately or fixed cells can be stored at 2-8˚C until analysing them. Analysis was done by FACS brand flow cytometer. Samples vortexed thoroughly prior to acquisition.

*Flow Cytometry Analysis:* The BD FACS Calibur (Becton, Dickinson Fluorescence Activated Cell Sorter is a trademark of Becton, Dickinson Company, CA, USA) had been used for immunophenotyping analysis. The BD FACS Calibur is a dual-laser, four-color, bench-top and fully integrated multiparameter system that is designed particularly to support a variety of applications.

The appropriate concentration for suspensions considered about $10^6$-$10^7$ cells/ml. The analysis was limited to the blast population, by gating on forward and side light scatter parameters therefore the majority of the lymphocytes, monocytes, and granulocytes were excluded. Analysis of presentation samples is assisted by the high percentage of blasts, but because normal hematopoietic progenitors also display the surface markers most commonly associated with AML (ie, CD34, CD38, CD33, CD13, and HLA-DR), the analysis of remission samples (4% blasts) is problematic, thus they were excluded from our study. We applied Acute Leukemia Panel with these following CD markers: CD2, CD3, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD45, CD61, GLYCO A, CD117, HLA-DR, cy MPO and TdT.

### *Statistical analysis*

Data were analysed qualitatively and quantitatively by means of SPSS 14 (Statistical package for social sciences 14). Frequency and descriptive analysis were used in all statistical process. The statistical significance value was chosen to be below 0.05.

## Results

Out of 56 cases with AML 33 (58.93%) cases were male and 23 (41.07%) of them were female. AML is more prevalent in men than women; male to female ratio was 1.5/1.0. Patient's age ranged between 20 and 85 (mean+/- SD: 40.7+/- 1.53). Based on patient's age, we divided them into four groups: younger than 30 years, from 31 to 50, between 41 and 50 and older than 50. AML was more prevalent in the fourth group.

Among different markers, the most positive markers in myeloid lineage antigens were CD38, CD45 (100%), CD13 (92.9), CD33 (90.2%), and MPO (42.3%) as well as hematopoietic progenitor cell surface antigens including HLA-DR (72%), CD117 (78.4%), CD34 (52%).

In acute myeloid leukemia, we considered lymphoid antigens as aberrant antigens. CD7 was positive in 72.7% of cases in M1 and 28.5% in M2 but M3 and M4 cases lacked this marker. We detected CD2, T-cell marker, in 58.35 of M1cases, 21.40% of M2 cases, 33.3 of M3 and 20% of M5; but M4 patients lacked this marker. (Table 1) The CBC analysis demonstrated a wide range of haemoglobin concentration, Platelet and WBC count which varied from normal to anaemia, thrombocytopenia to thrombocytosis and leukopenia to hyper leukocytosis. The status of remission and aberrant marker was illustrated in Table 2.

**Table 1.** Frequency of lymphoid marker in subclass of AML patient in FAB study

| FAB | T CELL MARKER | | | B CELL MARKER | | | |
|---|---|---|---|---|---|---|---|
| | CD2 | CD3 | CD7 | CD10 | CD19 | CD20 | CD22 |
| M0 | 1 | 1 | 1 | 1 | 1 | 2 | 3 |
| M1 | 7 | 5 | 8 | 2 | 3 | 0 | 2 |
| M2 | 3 | 1 | 4 | 0 | 1 | 0 | 0 |
| M3 | 3 | 0 | 0 | 0 | 0 | 0 | 1 |
| M4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M5 | 1 | 1 | 1 | 0 | 0 | 1 | 0 |
| M7 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |

**Table 2.** Aberrant marker and remission statues

| Positive aberrant marker | Remission status | | |
|---|---|---|---|
| | Remission | Not Remission | Total |
| CD2 | 5 | 10 | 15 |
| CD3 | 3 | 5 | 8 |
| CD7 | 6 | 10 | 16 |
| CD10 | 0 | 3 | 3 |
| CD19 | 1 | 5 | 6 |
| CD20 | 1 | 2 | 3 |
| CD22 | 1 | 5 | 6 |

### *Descriptive statistics for the variables of acute myelogenous leukemia.*

The WBC ranged from (430 - 2x105/µL) with a median of 8500/µL. The median and range for haemoglobin was 8gm/dl (5.2 – 14gm/dl), and the platelet count range was from (3600 – 8.8x104/µL) with a median of 3.95x105/µL. (Table 3)

**Tabel 3.** Patient characteristics in this study

| Characteristics | Range | Number |
|---|---|---|
| AGE | ≤30 | 17( 30.4%) |
| | 31-40 | 14(25%) |
| | 41-50 | 7(12.5%) |
| | 51≤ | 18(32.1%) |
| Hb | <10 | 39(69.6%) |
| | 10≤ | 17(30.4%) |
| WBC | <10000 | 34(60.7%) |
| | 10000≤ | 22(39.3%) |
| PLT | <20000 | 13(23.2%) |
| | 20000≤ | 43(76.8%) |
| Bone marrow Blast | <70% | 55(98.2%) |
| | 70%≤ | 1(1.8%) |

## Discussion

Human acute leukemias are broadly described by differentiation into myeloid or lymphoid lineages according to the expression of surface and/or cytoplasmic markers associated with their normal myeloid or B-cell and T-cell counterparts.[15] Immunophenotyping is a convenient method for quick

and reproducible diagnosis of the majority of hematological malignancies.[16] Aberrant phenotype in AML is known as a poor prognostic indicator.[17]

In the present study 56 new cases of AML for aberrant expression of lymphoid antigens were investigated. The presence of aberrant markers more than 20%, considered as cut-off value for aberrant phenotype definition. In the under study group, the ratio of male to female equals 1.5 to 1.0, which is similar with other ratio reported from international study.[18] Although, most prior studies emphasize that AML is more prevalent in patients over 60 years old in general populations,[19] our finding shows two age peak incidence for AML (≤30 and 51≤ years old).

Most frequently expressed lymphoid antigens were CD7 (31%) and CD2 (29%); and other marker was expressed as: CD3 (15.7%), CD19 (12.2%) CD22 (12.2%), CD20 (6.1%). This finding is in contrast with El-Sissy et al that they showed that CD9 (29.4%) has highest frequency in AML cases.[15] In another study, Bharat Bhushan et al noteded that the frequency of CD7 expression was lower than CD19 expression in AML.[20] These findings of the current study are consistent with those of Bahia, Shen and John et al who found CD7 was the most frequent lymphoid antigen expressed in AML.[11,21,22] In this research, FAB studies on AML patient showed that AML-M2 was the most common subtype of AML, which is in agreement with Lowenberg H findings.[19] Another important finding was that CD7 aberrant phenotype includes incidence in M1 (72.7 %) and in M2 (28.5), which in contrast with Bharat Bhushan who noted that CD7 had high occurrence in M5.[20] It has been investigated that CD2 is the most commonly expressed lymphoid antigen followed by CD7, however the previous study focuses on CD19 expression following CD7[20] and El-Sissy et al noted CD7 is afterward respect to CD9.[15] Recently, aberrant phenotype has been used to stage and monitor minimal residual disease (MRD) in acute myeloid leukemia.[23] This experiment did not detect any significant correlation between aberrant phenotypes and MRD in leukemic patients. Even though prior research demonstrate that the aberrant phenotype cannot be used as criterion to predict the relapse and remission of leukemia,[24] A strong relationship between aberrant phenotype and MRD has been noted in the literature.[25]

## Conclusion

Our findings showed that CD7 and CD2 were the most common aberrant marker in Iranian patients with AML. However, we are not find any significant correlation between aberrant phenotype changing and MRD in our population. Taken together, this findings help to provide new insights in to the investigation of other aberrant phenotypes that may play roles in diagnosis and therapeutic of AML.

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## Conflict of Interest

The authors report no conflicts of interest.

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