ABSTRACT

Background: Immunophenotyping improves both accuracy and reproducibility of acute leukemia classification and is considered particularly useful for identifying aberrant lineage association of acute leukemia, biphenotypic and bilineal acute leukemia, as well as monitoring minimal residual disease. Some immunophenotypes correlate with cytogenetic abnormalities and prognosis.

The Aim of Our Study: Is to determine aberrant lymphoid antigen expression in Saudi acute myeloid leukemia (AML), correlate them with FAB subtypes, evaluate early surface markers CD7 and CD56, and to investigate the role of cytoplasmic CD79a (a B cell marker that is assigned a high score of 2.0 in the WHO classification).

Patients and Methods: Thirty four newly diagnosed AML cases were included in this study, 47% showed aberrant lymphoid antigen expression. CD9 was the most frequently expressed lymphoid antigen (29.4%) followed by CD7 & CD19 (11.8%), CD4 (8.8%) and CD22 (2.9%). CD9 was expressed in 3/6 (50%) of M3 cases, CD7 was expressed in 11.8% and was mostly confined to FAB M1 and M2 and associated with immature antigens CD34, HLA-DR and TdT. CD56 was expressed in 7/34 (20.6%) cases, three of these cases (42.9%) belonged to the monocytic group. CD56 was also detected in 2 cases with 11q23 rearrangement. CD56 was expressed in 2/7 (28.6%) M2 cases, and was associated with t (8;21) (q22;q22) together with CD19. Co-expression of CD56 and CD7 was detected in 2.9% of the cases, CD79a was expressed in one case together with CD19, diagnosed as acute biphenotypic leukemia, and was associated with t(8;21) (q22;q22).

Conclusion: Minimal residual disease in AML is very difficult to trace, detection of aberrant expression of lymphoid antigens will make it easier. The high score given to CD79a by EGIL is questionable based on cytogenetic classification.

Key Words: Immunophenotyping – Acute myeloid leukemia (AML) – Aberrant lymphoid antigen expression.

INTRODUCTION

Immunophenotyping improves both accuracy and reproducibility of acute leukemia classification and is considered particularly useful for identifying poorly differentiated subtypes of acute leukemia, acute myeloid leukemia (AML) with lymphoid marker expression and acute lymphatic leukemia (ALL) with myeloid marker expression. Immunological studies of leukemic blasts have become critical also for identifying biphenotypic and bilineal acute leukemias. At present, while the prognostic value of individual antigen expressions is still controversial, it is important in the immunologic detection of minimal residual disease, especially in AML, as it seems to be important in monitoring the acute leukemia patients in remission [1]. A new WHO classification has been recently used in the diagnosis of leukemia. Based on co-expression and correlation of lineage-associated antigens, multiparameter high-resolution flow cytometry has been developed to precisely identify lineage characteristics of leukemia. Some immunophenotypes correlate with cytogenetic abnormalities and prognosis [2]. Forty six percent (46%) of ALL cases and 48% of AML cases [3] were reported to have aberrant expression of a single antigen associated with another cell lineage, most commonly CD2 [3] and CD7 [4] in AML and CD33 in ALL [5,6].

CD56, a neural cell adhesion molecule, expressed on NK cells, and some T cell subsets,
is commonly expressed on various haematopoietic neoplasms, including clinically aggressive lymphoma and myeloma, also on a subset of AML. It can be used as a prognostic parameter in certain cases [7,8].

Acute leukemias that express antigens associated with more than one lineage have been classified as acute lymphocytic leukemia with myeloid markers, acute myeloid leukemia with lymphoid markers, or biphenotypic acute leukemia (BAL) [6].

An earlier scoring system by the European Group for the Immunological Characterization of Acute Leukemias (EGIL) [4] (Table 1) in which antigens detected by the flow cytometric immunophenotyping of cases were assigned a score of 2, 1, or 0.5 depending on the specificity of a particular antigen for myeloid, T-lymphoid, or B-lymphoid lineage. Cases having a score greater than two for both the myeloid and either the T- or B-cell lines are designated biphenotypic acute leukemias [4,9].

One marker, CD79a, is a cytoplasmic antigen that acts as a mediator of signal transduction from the cell surface to the cytoplasm in association with CD79b and is expressed early in B-cell development [10]. In the EGIL classification of biphenotypic acute leukemia, it is assigned a score of 2, suggesting that its expression is highly specific for B-cell lineage. Its expression is so specific for B-lymphoid lineage that some researchers have considered its presence in association with blast antigens to be indicative of B-ALL despite the co-expression of myeloid markers [11]. Cytogenetic abnormalities are commonly encountered in leukemias of ambiguous lineage, with t(9;22) (q34;q11.2) most frequently encountered. Less commonly, abnormalities in chromosome 11q are seen [9]. Leukemias of ambiguous lineage with cytogenetic abnormalities are believed to be associated with a poorer prognosis than are leukemias without demonstrable abnormalities [9].

**Aim of the study:**

Our aim is to determine aberrant lymphoid antigen expression in Saudi Acute myeloid leukemia, correlate them with FAB subtypes, evaluate of early markers CD7 and CD56, and to investigate the role of cytoplasmic CD79a, a B cell marker that is assigned a high score of 2.0 in the WHO classification.

**PATIENTS AND METHODS**

Thirty-four newly diagnosed adult AML cases presenting to King Fahd Medical City, Riyadh, Saudi Arabia from October 2004 till June 2006 were included in this study. Patients were subjected to:

A- Bone marrow morphological examination.

B- Flowcytometric analysis: The BD – FACS-Canto system (Becton Dickinson-Bioscience, USA) and a reagent system (BD-FACS set up, USA) were used to prepare bone marrow or whole blood samples for immunophenotypic analysis by flowcytometry. The panel of monoclonal antibodies used includes: Miscellaneous panel (CD34, CD45, CD56, HLADR, TdT). B cell panel (CD19, CD20, CD22, CD24, CD10, CD9, CD79a, surface and cytoplasmic immunoglobulin), T cell panel (CD2, CD5, CD7, CD3, CD4, CD8), myeloid panel (myeloperoxidase, CD13, CD33, CD14, CD15, CD11b, CD11c, CD117, CD64, CD65).

C- Cytogenetic analysis: Included preparation, banding and karyotyping technique of 24, 48, and 72 hours cultured cells according to the basic techniques of Moorhead et al. [12]. Chromosomal analysis and karyotyping: The chosen metaphases were photographed and analyzed using a computer image analyzer (Vysis Quips XL=Genetics work station) according to Paris Conference recommendations [13] and the International System of Human Cytogenetic Nomenclature (ISCN) [14] recommendations. For each case, 20 metaphases were analyzed to detect any chromosomal aberration.

**RESULTS**

Thirty-four cases, newly diagnosed as acute myeloid leukemia, were included in the study. Their age ranged from 16 to 60 years, with a mean of 32.4 years and median of 35 years. Males were 21 and females were 13, with a M:F=1.6/1. They were classified according to FAB classification into M1 (9 cases), M2 (10 cases), M3 (5 cases), M4 (2 cases), M5 (5 cases), M6 (1 case), M7 (2 cases). Aberrant lymphoid antigens were detected in 16/34 (47%) cases of which 11 cases showed CD34 expression. The five CD34 negative cases were mainly of FAB M3.
CD9 was expressed on 10/34 cases (29.4%), 3 cases FAB M3 subtype, 2 cases for each FAB M1, M2 and M5 subtypes and one case for FAB M7. CD19 was expressed on 4/34 cases (11.8%), 2 cases FAB M2, and one case for each FAB M1 and M6. CD4 was expressed on 3 (8.8%) cases, one case for each FAB M2, M3 and M5, while CD22 was expressed on one case FAB M1.

CD7 was co-expressed on 4/34 (11.8%) cases, 3/4 cases were FAB subtype M1 and 1/4 cases was FAB subtype M2. Their cytogenetics pattern showed normal karyotype, only one case of FAB M1 had t(10;11) (p12;q23) and all of them co-expressed CD34 (Table 3).

CD56 was expressed on 7/34 (20.6%) cases, 2/7 cases were FAB M5, 2/7 cases were FAB M2, 1/7 was FAB M0, 1/7 was FAB M1 and 1/7 cases was FAB M4 and was negative in all the remaining cases. CD56 was associated with t(8;21) (q22;q22) in both M2 cases and was associated with 11q23 rearrangement in two cases, FAB M1 and M5 (Table 4).

Only one case of FAB M1 showed co-expression of CD7 and CD56 having 11q23 rearrangement (Table 5).

CD79a was expressed on only one case classified as biphenotypic acute leukemia, score (3) according to EGIL scoring system, and was associated with t(8;21) (q22;q22) (Table 6).

Table (1): Scoring system for markers proposed by the European Group for the immunologic classification of leukemia (EGIL).

<table>
<thead>
<tr>
<th>Score</th>
<th>B-Lymphoid</th>
<th>T-Lymphoid</th>
<th>M-Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Cyt CD97a, Cyt, IgM, Cyt CD22</td>
<td>CD3m/Cyt, Anti-TCR</td>
<td>MPO</td>
</tr>
<tr>
<td>1</td>
<td>CD19, CD20, CD10</td>
<td>CD2, CD5, CD8, CD10</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>Tdt, CD24</td>
<td>TdT, CD7, CD1a</td>
<td>CD14, CD15, CD64</td>
</tr>
</tbody>
</table>


Table (2): CD9, CD19, CD22 and CD4 expression (n=34).

<table>
<thead>
<tr>
<th>FAB</th>
<th>CD9</th>
<th>CD19</th>
<th>CD22</th>
<th>CD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>M2</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>M3</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>M4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M5</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>M6</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M7</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table (3): CD7 expression, relation to cytogenetics and CD34.

<table>
<thead>
<tr>
<th>FAB</th>
<th>CD7</th>
<th>Karyotype</th>
<th>CD34</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>3 cases</td>
<td>46; XY, 46; XY, 46; XY; t(10;11) (p12;q23)</td>
<td>Positive</td>
</tr>
<tr>
<td>M1</td>
<td>1 case</td>
<td>46; XY</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table (4): CD56 expression, relation to cytogenetics.

<table>
<thead>
<tr>
<th>FAB</th>
<th>CD56</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>1 case</td>
<td>48; XY; t(1;7) (q21;q22), +9 +13, del (17) (p11.2)</td>
</tr>
<tr>
<td>M1</td>
<td>1 case</td>
<td>46; XX; t(10;11) (p12;q23)</td>
</tr>
<tr>
<td>M2</td>
<td>2 cases</td>
<td>46; XX; t(8;21) (q22;q22)</td>
</tr>
<tr>
<td>M4</td>
<td>1 case</td>
<td>46; XX; t(8;21) (q22;q22)</td>
</tr>
<tr>
<td>M5</td>
<td>2 cases</td>
<td>46; XX; t(19;11) (p22;q23)</td>
</tr>
</tbody>
</table>

Table (5): Co-expression of CD7 and CD56.

<table>
<thead>
<tr>
<th>FAB</th>
<th>CD7</th>
<th>CD56</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Positive</td>
<td>Positive</td>
<td>46; XY; t(10;11) (p12;q23)</td>
</tr>
</tbody>
</table>
DISCUSSION

Human acute leukemias are broadly defined as having myeloid or lymphoid differentiation according to the expression of surface and/or cytoplasmic antigens associated with their normal myeloid or B/T-lymphoid counterparts. Aberrant antigen expression in AML is recognized as a poor prognostic indicator (2). Among 34 newly diagnosed AML cases in our study, 47% showed aberrant lymphoid antigen expression compared to 48% as reported by Khalidi et al. [15] and John et al. [16] and 30% as reported by Zhu et al. [2].

In our study, CD9 was the most frequently expressed lymphoid antigen (29.4%) followed by CD7 & CD19 (11.8%), CD4 (8.8%) and CD22 (2.9%), in contrast to the results of Bahia et al. [17] who reported that CD7 was the most frequent lymphoid associated antigen in their AML cases. The same results were reported by Shen et al. [18], where CD7 was expressed in (12.8%), followed by CD19 (6.4%) and CD2 (5.1%) in AML cases. John et al. [16] reported that CD2 and CD7 were the most common lymphoid antigens associated with AML.

Among 6 cases of FAB M3 morphology with t(15;17) included in this study, 3 cases expressed CD9. The results are in contrast to those reported by Wang et al. [19], where no co-expression of lymphoid antigens was detected among 7 M3 cases with (15;17).

CD7 antigen, a T-cell lineage associated antigen, is expressed in a minority of patients with AML. CD7 was expressed in 4/34 (11.8%) of our cases, compared to 15/40 (37.5%) reported by Macedo et al. [20], 6/60 cases (10%) reported by Saxena et al. [21], also in 79/256 (30.9%) by Orgata et al. [22], 7/40 (17.5%), reported by Zhu et al. [2], 28/222 (12.6%) reported by Shen et al. [18] and 15/46 (32.6%) reported by Juluis et al. [8]. In our study, CD7 was mostly confined to FAB M1 and M2 and was associated with the immature antigens CD34, HLA-DR and TdT, in agreement with Saxena et al. [21]. Juluis et al. [8] reported that out of 10 cases diagnosed as acute myeloblastic leukemia (FAB M0, M1, M2), 8 cases expressed CD7. Our results are in agreement with other international reports [8,21,23] which supports that CD7-positive AML originates from early hematopoietic precursors and indicates biologic aggression in a significant proportion of patient.

In our cases, CD7 was mostly associated with normal karyotype, except for one case with t(10;11) (p12;q23), in contrast to that reported by Orgata et al. [22], who found that the proportion of CD7+ cases increased stepwise from the cases with favorable cytogenetics to the cases with intermediate and unfavorable cytogenetics (3 out of 69 cases, 51 out of 140 cases and 25 out of 47 cases respectively, p<0.0001), and CD7-positivity adversely affected the survival only in the cases with unfavorable cytogenetics (p<0.03). They recommend that CD7 expression in AML should be interpreted in association with the cytogenetics.

Among our study cases, CD56 was expressed in 7/34 cases, (20.6%), in agreement with Shen et al. [18] who reported that CD56 was present in 84/222 case (37.8%). Juluis et al. [8] reported that CD56 expression was found in 21.7% of their AML cases and Ma et al. [24] reported 13.2%, CD56 expression among 297 AML cases. Forty-three percent (3/7) of our cases belonged to the monocytic group, in agreement with Graf et al. [25] and Wang et al. [19] who reported that CD56 was mainly expressed in M5 cases. In our study, CD56 was also detected in 2 cases with 11q23 rearrangement, this may explain the high association with M5 cases. CD56 was expressed in 5/27 (28.6%) M2 cases, and was associated with t(8;21) (q22;q22) together with CD19, in agreement with Hurwitz et al. [26] reported that co-expression of CD19 and CD56 in their cases was found only in the t(8;21) group (9/16 M2 cases). Furthermore,

Table (6): CD79a expression, relation to cytogenetics.

<table>
<thead>
<tr>
<th>Biphenotypic</th>
<th>CD79a</th>
<th>Cytogenetics</th>
<th>Immunophenotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemia</td>
<td>1 case</td>
<td>46; XX; t(8;21) (q22;q22)</td>
<td>CD45, CD34, CD13, CD33, MPO, CD117, CD79a, CD19</td>
</tr>
</tbody>
</table>

Aberrant Lymphoid Antigens in AML
this phenotype was not found in 48 evaluable cases of de novo AML of the FAB M1, M3, M4, M5, or M7 subtypes. Wang et al. [19] reported 10 cases with t(8;21) (q22;q22) with high expression of CD56, CD19. On the other hand Graf et al. [25] concluded that CD56 positive subtypes of AML seem to be a separate entity with a worse prognosis independent of the karyotype.

Co-expression CD56 and CD7 was detected in (2.9%) of our cases compared to (13%) reported by Juluis et al. [8].

CD79a was expressed in one case together with CD19 and was diagnosed as acute biphenotypic leukemia (score 3) according to EGIL scoring, and with t(18;21) (q22;q22). Koslov et al. [6] reported 2 cases diagnosed as acute biphenotypic leukemia expressing CD79a together with CD19 and carrying t(8;21) (q22;q22). Both patients achieved remission as did one patient having the same criteria reported by Killck et al. [27]. Scolnik et al. [28] reported one case with CD79a positive biphenotypic acute leukemia having t(15;17) (PML/RARA) rearrangement who stayed in remission for 3 years.

Although immunophenotyping has occupied a dominant role in the diagnosis of acute leukemia, but currently subtypes of AML and ALL are classified and their prognosis determined on the basis of cytogenetic abnormalities [5,29]. It is likely that the chromosomal rearrangement will give a better indication of prognosis than immunophenotypic ambiguity. Emphasizing the primary role that cytogenetics plays in the classification of leukemia and questioning the high score given by EGIL for CD79a, we believe, in agreement with these authors, that the leukemias reported here do not show lineage ambiguity but rather represent AML having an aberrant expression of B-lymphoid antigens. It is doubtful that such cases should be considered acute biphenotypic leukemia.

**Conclusion:**

Minimal residual disease in AML is very difficult to trace, detection of aberrant expression of lymphoid antigens will make it easier. Aberrant lymphoid antigens were detected in 47% among Saudi acute myeloid leukemia cases. CD9 is the most commonly expressed aberrant lymphoid antigen. CD7 is confined to acute myeloblastic leukemia. CD56 is associated with t(8;21) (q22;q22) and 11q23 rearrangement. The high score given to CD79a by EGIL is questionable based on cytogenetic classification. Further studies with larger numbers of patients are recommended to confirm our results.

**REFERENCES**


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