Prevalence of uncommitted antigens and lymphoid markers in acute myeloid Leukemia

Dr Fareed Th. Haddad* MD
QRMH, Pediatric hemato/oncology group, P.O. Box Amman 11941 AL-Jubaha 1992

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ABSTRACT

Flow cytometry is a well known investigation for detecting the cluster of differentiation (CD antigens) in leukemic cells. It has the capability in a short time to measure multiple parameters on each single cell, which is sufficient to diagnose types, subtypes and even estimate to some the future prognoses. Objective: To evaluate Prevalence of uncommitted antigens and lymphoid markers in acute myeloid Leukemia flow cytometric results for a patient’s diagnosis of acute myeloid leukemia.

Method and material: Results of flow cytometry for 163 patients, between January 2006 and December 2011 who were newly diagnosed as having acute myeloid leukemia (AML) were retrospectively revised, 110 patients were male and 53 patients were female with male to female ratio close to 2:1. And age distribution as 35 pediatric patients in comparison to 128 adult patients.

Results: Most of patients were adult (128/163 patients (78.5%), and 110/163 (67.5%) were males. Among all AML Among all AML results showed 6 patients M0(3.7%), 32 patients M1 (19.6%), 47 patients M2 (28.8%), 15 patients M3 (9.2%), 38 patients M4 (23.3%),15 patients M5(9.2%),4 patients M6(2.5%), and 6 patients (3.7%)M7. In our study 6.2% of AML pts showed negative for myeloperoxidase, CD34 negative 48/141 pts (34%), and CD117 negative 21/142 pts (14.8%). while CD7 was positive in 28/130 (21.5%).

Conclusion: Flow cytometry is an informative test in diagnosis and identify AML subtypes. Having single uncommitted Antigens may suggest less differentiation and Positive lymphoid Antigens marker (especially CD7) in AML patients do not change their diagnosis, although make them more badly prognosis phenotypically. Negative myeloperoxidase is not against diagnosis of AML.

1. Introduction

As it is derived from its name cyto means cell, metry represents measurement. For that flow cytometry is routinely used in diagnosis of health disorders, especially blood malignancy but has many other applications in both research and clinical practice. A common variant is to physically sort particles based on their properties, so as to purify populations of interest1, 2.

Our normal cells express a variety of cell surface markers, depends on its type and degree of maturation. However, abnormal growth of patient’s cells may interfere with the natural expression of markers resulting in over expression of some and under-representation of others. Flow cytometry can distinguish between healthy and diseased cells. Flow cytometry is used to aid the diagnosis counting, examining, detection of minimal residual leukemia after therapy and sorting microscopic cell particles of acute leukemia or non-Hodgkin’s lymphoma in addition to other major applications of FCM [1, 2].

AML is the most common acute leukemia in adults and accounts for approximately 80 percent of adults cases. In contrast, AML accounts for less than 10 percent of acute leukemias in children less than 10 years of age [1, 2].

We aim to focus on prevalence of uncommitted Antigens as primitive stem cell markers in AML. And light on significance of lymphoid markers on AML clinical picture.
One hundred and sixty three samples of bone marrow (BM) and/or peripheral blood (PB), diagnosed as acute myeloid leukemia AML, were revised in a retrograde manner for their flow cytometry results at onset of disease before chemotherapy treatment and without being in relapse. These results were collected between January/2006-december/2011 over different age groups at King Hussein medical center (KHMC) in Jordan.

Age and sex were included in our study, and 20% level was considered as cut-off point for positivity except for myeloperoxidase antigen where >3% was the limit. Flow cytometry analysis using two-color instrument was performed, after proper sampling and professional high standard of preparations.

CD markers of MPO, CD13, and CD33 were studied as diagnoses evidence for acute myeloid leukemia AML. But we also revised others like CD34, HLA-DR, CD117, TdT as uncommitted antigens and (CD10, CD19, CD7, and CD2) as lymphoid markers.

The immunophenotypic results for AML patients as seen in table II, showed the followings, 137/146 patients (93.4%) were MPO positive [9/146(6.6%) MPO-VE], 135/152 patients (88.8%) were CD33 positive, and 83/98 patients represent (84.7%) were positive for CD13.

Out of all, 6 patients (3.7%) were M0, most of M0 5/6 were males adults, 32 patients (19.6%) were M1, 47 patients (28.8%) were M2 which was the commonest subtype, 15 patients (9.2%) were M3 almost all M3 were adults with equal sex distribution, 38 patients (23.2%) were M4 with three forth of them were male adults, 15 patients (9.2%) were M5, four patients (2.4%) were M6, 6 patients (3.7%) were M7.

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Acute myeloid leukemia patients showed CD34 positive in 66% of patients, CD117 positive in 85.2% of patients, HLA-DR positive in 72.5% of patients, TdT positive in 3/132 represents 2.3% of patients. And CD7 positive in 28/130 of patients represents 21.5%.

Otherwise, almost all AML FCM results were negative for other lymphoid antigen markers, CD10 and/or CD19 and/or CD2.
Table-II The immunophenotypic results for AML patients

<table>
<thead>
<tr>
<th>CD</th>
<th>Status</th>
<th>Results</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>positive</td>
<td>137/146</td>
<td>93.8%</td>
</tr>
<tr>
<td>Cd33</td>
<td>positive</td>
<td>135/152</td>
<td>88.8%</td>
</tr>
<tr>
<td>Cd13</td>
<td>positive</td>
<td>83/98</td>
<td>84.7%</td>
</tr>
<tr>
<td>Cd34</td>
<td>positive</td>
<td>93/141</td>
<td>66%</td>
</tr>
<tr>
<td>HLA DR</td>
<td>positive</td>
<td>108/149</td>
<td>72.5%</td>
</tr>
<tr>
<td>Cd117</td>
<td>positive</td>
<td>121/142</td>
<td>85.2%</td>
</tr>
<tr>
<td>TDT</td>
<td>negative</td>
<td>129/132</td>
<td>97.7%</td>
</tr>
<tr>
<td>Cd10</td>
<td>negative</td>
<td>36/36</td>
<td>100%</td>
</tr>
<tr>
<td>Cd19</td>
<td>negative</td>
<td>89/90</td>
<td>98.9%</td>
</tr>
<tr>
<td>CD7</td>
<td>negative</td>
<td>102/130</td>
<td>78.5%</td>
</tr>
<tr>
<td>CD7</td>
<td>positive</td>
<td>28/130</td>
<td>21.5%</td>
</tr>
<tr>
<td>CD2</td>
<td>negative</td>
<td>102/105</td>
<td>97.1%</td>
</tr>
</tbody>
</table>

4. Discussion

Over the past few years, many studies have indicated that immunological markers, particularly those concerning cell surface antigens, may be highly discriminating with respect to cellular identity in the hemopoietic system. We reviewed many articles whose authors did retrograde revision for their pts previous result over years. Each one discusses that from his point of interest.

Multicolor flow cytometry FCM provides the opportunity to evaluate multiple antigens simultaneously, making it possible to characterize various cell populations in a more precise manner [1, 2] that ease differentiation AML from ALL and its subtypes. In our study M1 and M2 represent around 50% of all pts.

AML blasts can be identified by any of the following ways [3]:

- Presence of an Auer rod on microscopy [3]
- Positivity for Sudan black B, myeloperoxidase, nonspecific or chloroacetate esterase [3].

A myeloperoxidase MPO done easily within few minutes, and simply determine the presence of myeloid blasts. But MPO negative does not rule out AML, since minority of cases are negative. In our study 6.2% of AML pts showed negative for myeloperoxidase.

Flow cytometry identifying the expression of myeloid antigens

Specific cytogenetic abnormalities that are seen only in myeloid leukemias even if BM blasts not exceed 20% cut off point [3].

In AML FCM it mentioned that up to 20 percent of acute leukemias will demonstrate biphenoletic or mixed lineage with expression of both myeloid and lymphoid markers. Myeloblasts may express T or B cell antigens, most commonly in cytogenetically defined subtypes of AML [3].

According to Peter H. Wiernik in Neoplastic Diseases of the Blood a CD markers overlap was obvious, between myeloid, stem cell, and lymphoid cells. (Diagram II). We focus here on uncommitted antigens CD34, CD117, TdT, HLA-DR, and other overlap antigen like CD7 lymphoid Ag[4].

Diagram II Antigenic overlap between cells lineages

Peter H. Wiernik and others described in their article in British Journal of Hematology, 1998, 100, 265-272 the uncommitted antigens. As listed in table-III [5].

Bold style CD markers of the table-III were our study interest, myeloid one as base of diagnoses, lymphoid positivity in this myeloid lineage leukemias, and lastly uncommitted antigens.

Table-III uncommitted and other known antigens list

<table>
<thead>
<tr>
<th>Uncommitted Ag</th>
<th>Lymphoid Ag</th>
<th>Myeloid Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>TdT</td>
<td>CD56</td>
<td>CD3 (c &amp; m)</td>
</tr>
<tr>
<td>CD34, CD117</td>
<td>CD122</td>
<td>CD7</td>
</tr>
<tr>
<td>CD117</td>
<td>CD58</td>
<td>CD10</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>CD38</td>
<td>CD19</td>
</tr>
<tr>
<td>CD11a</td>
<td>CD54</td>
<td>CD20</td>
</tr>
<tr>
<td>Cd25</td>
<td>p-glycoprotein</td>
<td>CD22 (c &amp; m)</td>
</tr>
<tr>
<td></td>
<td>CD24</td>
<td>CD15s</td>
</tr>
</tbody>
</table>

Many Lineage-uncommitted antigens are stem cell antigens, which in normal hematopoiesis mark cells with self renewal capacity and differentiation potential along more than one cell lineage. During normal hematopoiesis, there diminishes parallel to progressive maturation. When present on leukemic blast cells, they may suggest a less differentiated phenotype. CD34, CD117, TdT, CD HLA-DR[4, 5].
Each one sporadically, may carry specific effect on prognoses, for example TdT which shows equivocal results on prognoses, many studies have found poor prognosis of AML with TdT expression, and other showed better prognosis [6].

In Canadian study they talk about primitive AML cells capable of long-term proliferations which differ from primitive normal progenitor cells in their lack of CD34 and/or CD117 surface expression [8]. It is found in our study that CD34 negative were seen in 48/141 pts (34%), and CD117 negative in 21/142 pts (14.8%).

Others like CD7 positivity in AML fulfilling the FAB criteria showed unique and characteristic clinical manifestations. As mentioned by Kita and other investigator patients with CD7 positive AML were younger males with higher incidence CNS involvement, and carry unfavorable prognoses due to poor respond to standard therapy, than CD7 negative AML patients [7]. Also CD7 positivity considered to be a phenotypically risk factor for AML, that even showed a different clinical, in their study CD7 was positive in 49/154 cases (31.8%) [9]. While Asvadi Kermani, M D found in his study [10] CD7 as the most common T- lymphoid markers in AML patients, it was (17%), and seen mostly in M1 and M2 AML subgroups. While we found 28/130 CD7 positive, which represents (21.5%) in our study.

5. Conclusion
Flow cytometry is rapid and fast technique for diagnosis and identifying AML subtypes.

Presence of uncommitted markers in AML flow will not change the diagnosis, but it may suggest less differentiation AML leukemia.

Negative myeloperoxidase MPO is not against AML diagnoses.

CD7 positivity considered to be a phenotypically risk factor for AML which may have unfavorable and specific presentation.

6. References
[8] Primitive acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo lack surface expression of c-kit (CD117) Allison Blaira and Heather J. Sutherland, baTerry Fox Laboratory, British Columbia Cancer Agency, Vancouver Hospital and Health Sciences Centre, Vancouver, BC, Canada; b Department of Medicine, University of BC, Vancouver, BC, Canada (Received 24 September 1999; revised 7 January 2000; accepted 9 February 2000)
[10] Immunophenotyping of Acute Leukemia in Northwestern Iran IJMS Vol 27, No. 3, September 2002. I.Asvadi Kermani, M D, Shahid Ghazi Hospital, Tabriz University of Medical Sciences, Tabriz, Iran