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Original Article

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/ out of 163 patients (78.5%), and 110/163 (67.5%) were males. Among all AML Among all AML results showed 6patients 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.

* Corresponding Author : Dr Fareed Th. Haddad* MD
QRMH, Pediatric hemato/oncology group.
P.O. Box Amman 11941 AL-Jubaha 1992
Mobile # 00962775543925
00962796359850
E-mail: fareed36@hotmail.com

2. Materials and Methods

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 deferent 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 stander 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

3.Results:

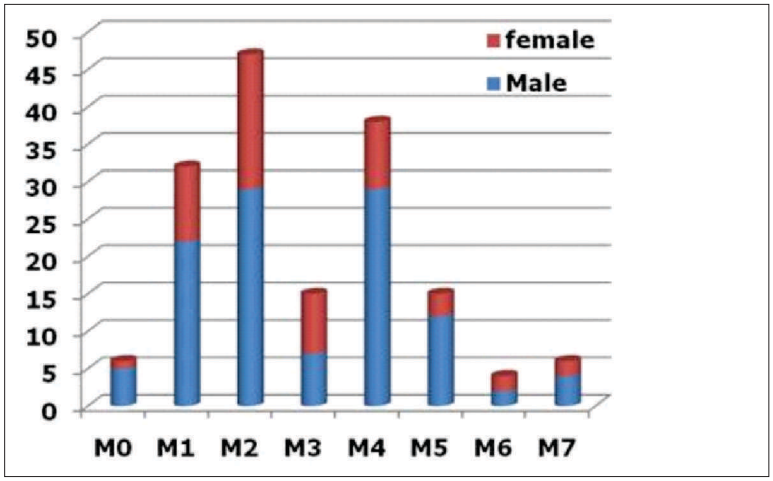
The revision for our 163 patients flow cytometry results showed; 110 males and 53 females with ratio of male: female close to 2:1. The age distribution was: 128 patients were adults and 35 patients at pediatric age group, with adult: children ratio 3.6: 1. (Table 1) and (diagram 1)

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 adult 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. (Table 1) and (diagram 1)

Table 1 showed AML subtypes, age groups, and gender distribution.

		M0	M1	M2	M3	M4	M5	M6	M7	Total
No. of pts		6	32	47	15	38	15	4	6	163
Age groups	Peds	1	5	13	1	10	2	1	4	35
	Adult	5	27	34	14	28	13	3	2	128
sex	Male	5	22	29	7	29	12	2	4	110
	Female	1	10	18	8	9	3	2	2	53

Diagram I shows AML subtypes and its gender distribution



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.

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

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

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 represents around 50% of all pts.

AML blasts can be identified by any of the following way [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 biphenotypic 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 CDs 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] .

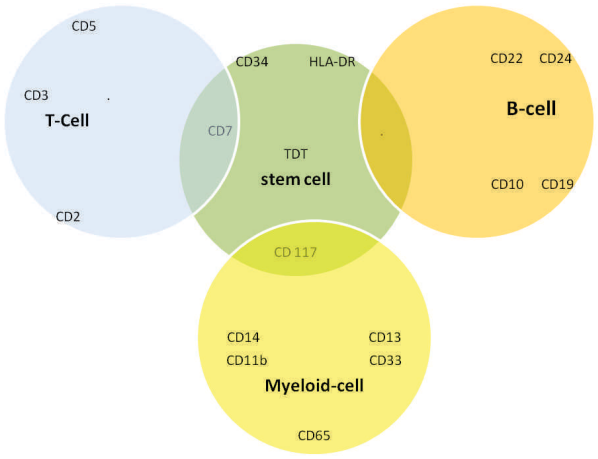


Diagram II Antigenic overlap between cells lineages

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

Uncommitted Ag		lymphoid Ag	myeloid Ag
TdT	CD 56	Cd3 (c & m)	MPO
CD34,	CD122	CD7	CD33
CD117	CD58	CD10	CD13
HLA-DR	CD38	CD19	CD11b
CD11a	CD54	CD20	CD14
Cd25	p-glycoprotein	CD22 c & m	CD15
		CD24	CD15s
			lactoferrin

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 found28/130 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.

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