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Research Article

Aberrant Antigen Expression in Patients with Acute Leukemia

Gamal Abdul Hamid[1]* and Mayson Awadh Akrabi[2]

[1]*Faculty of Medicine, Aden University, Aden, Yemen*

[2]*National Oncology Center, Aden, Yemen*

***Corresponding Author:** Gamal Abdul Hamid, Professor, Faculty of Medicine, Aden University, Aden, Yemen.*

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Abstract

Objective: To assess the frequency of aberrant antigens expression in acute leukemias.

Materials and Methods: A descriptive study of acute leukemia cases was conducted at National Oncology Center Aden in Al-Sadaka Teaching Hospital over one year (January 2015 to June 2016). A total of 55 cases of acute leukemia diagnosed by multi parameter flowcytometry performed on peripheral blood and/or fresh bone marrow aspirates. The co-expression of myeloid markers on lymphoblasts and lymphoid markers on myeloblasts was analyzed.

Results: 55 cases of acute leukemias were retrieved; 29 (52.7%) of them were acute lymphoblastic leukemias, were B cell type (n = 27) more than T cell type (n = 2) and the remainder 26 (47.3%) acute myeloblastic leukemias. proved by flow cytometry to be AML subtypes and one was AML with mixed phenotype (biphenotypic). Aberrant immunophenotype expression was observed in (43%) AML cases and in (32%) of ALL cases. CD19 and CD7 were the commonest aberrant lymphoid marker expressed in AML which was noted in 30.8 %,26.9% respectively Aberrant expression included CD4 in 15.4% of AML patients. CD20 15.4%, CD79a and CD10.5%. The most aberrant myeloid marker expressed in ALL which was CD13, CD33 20.7% for each followed by CD117 10.3%.

Conclusion: The incidence of aberrant antigen expression in acute leukemia was comparable with most published international data. In this study CD19 and CD7 were the commonest aberrant lymphoid marker expressed in AML. Further studies are needed to confirm the correlation between aberrant phenotypes with prognosis and therapeutic response of acute leukemia.

Keywords: *Aberrant Phenotypes; Acute Leukemia; Flow Cytometry*

Introduction

Acute leukemias are a group of neoplastic disorder with varying clinical, morphologic, immunologic, and molecular characteristics [1]. Acute leukaemias are characterized by proliferation and accumulation of immature hematopoietic cells in peripheral blood and/or bone marrow; since cells of the leukaemic clone continue to proliferate without maturing to end cells and dying there is continued expansion of the leukaemic clone and immature cells dominate.

Immunophenotype by Flow cytometry is useful method for diagnosis, staging, classification and monitoring of acute leukemia.

Materials and Methods

A descriptive study of acute leukemia cases was conducted at National Oncology Center Aden in Al-Sadaka Teaching Hospital over one year (January 2015 to June 2016). A total of 55 cases of acute leukemia diagnosed by multi parameter flow cytometry performed on peripheral blood and/or fresh bone marrow aspirates. The co-expression of myeloid markers on lymphoblasts and lymphoid markers on myeloblasts was analyzed.

Bone marrow aspirates or PB samples were collected on EDTA tubes and immediately transported to the flowcytometry laboratory. For surface antigen staining, the received samples were lysed using homemade lysing solution (8g Ammonium chloride, 1g EDTA, and 0.1g dihydrogen potassium phosphate), washed with phosphate buffered saline (PBS) until complete RBCs lysis and resuspended in appropriate amount of PBS. The cells were stained with different fluorescently labeled monoclonal antibodies (mAbs) according to manufacturer recommendations (Dakocytomation, Denmark, and Beckman Coulter, Germany). One hundred micro liters of cell suspension were mixed with 10 µl of the fluorescently labeled mAb and incubated in the dark at room temperature (RT) for 30 minutes. Washing with PBS containing 2% bovine serum albumin was done twice and the pellet was resuspended in PBS and analyzed immediately on flow cytometer. For detection of cytoplasmic and nuclear antigens, Intra Prep Permealization Kit was used (Beckman Coulter, Germany). Fifty microliters of EDTA PB/BMA sample were mixed with 100 µl of IntraPrep reagent 1 (fixative), incubated for 15 minutes at RT protected from light and washed with PBS. 100 µl of IntraPrep reagent 2 (permealization) were mixed with the cells and incubated for 5 minutes at RT without vortexing nor shaking. The tube was shook carefully and manually for 2 - 3s and then 10 - 20 µl of the mAb were added, vortexed, and incubated for 20 minutes in case of cytoplasmic antigens and for 1h in case of nuclear antigens at RT protected from light. Then, the mixture was washed and resuspended in PBS and analyzed on the flowcytometer immediately. Different combination of mAb against the following antigens were used: CD1a, cCD3, cIgM, CD79a, cMPO, CD34, CD3, CD4, CD5, CD7, CD8, TdT, CD10, CD19, CD20, CD13, CD33, CD14, HLADR and CD117.

The cells were analyzed with the most appropriate blast gate using the combination of forward and side scatters. Many of the cases were gated on CD45dim versus side scatter to isolate the blast population, and 10000 list mode events were collected on the blast gate. A positive signal was recorded if 20% or more of the cells reacted with the given monoclonal antibody. Samples that gave a positive signal on < 20% cells were recorded as 'low expression; however, the arbitrary cutoff limit for a positive TdT expression was 10% or more. The mAbs were used in different combinations of fluorochromes; namely fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin-cyanine5 (PeCY5).

The collected data was introduced into the SPSS program (version 20), the analysis data tabulated in statistical tables. Descriptive statistics comprises frequency, mean, standard deviation and percentages.

Results

The studied patients were 55 acute leukemias, 29 (52.7%) of them were acute lymphoblastic leukemias and the remainder 26 (47.3%) acute myeloblastic leukemias. Statistically, there is no significant difference between the percentages of ALL versus AML ($p > 0.05$).

Cluster of differentiation	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
Markers of progenitors						
CD34	22	84.6	16	55.2	38	69.1
HLA-DR	10	38.5	10	34.5	20	36.4
CD117	14	53.8	3	10.3	17	30.9
TdT	0	0.0	4	13.8	4	7.3
B lineage markers						
CD19	8	30.8	24	82.8	32	58.2
CD22	1	3.8	8	27.6	9	16.4
CD20	4	15.4	11	37.9	15	27.3
CD79a	3	11.5	16	55.2	19	34.5
CD10	3	11.5	22	75.9	25	45.5
cIg M	1	3.8	3	10.3	4	7.3
T lineage markers						
CD7	7	26.9	2	6.9	9	16.4
cCD3*	0	0.0	2	6.9	2	3.6
CD2	1	3.8	1	3.4	2	3.6

Table 1: *Results of progenitors and lymphocytic cluster of differentiation in patients with acute leukemia.*
**c: Cytoplasmic.*

This table showed that markers of progenitors were expressed more in AML more than in ALL blast cells, except TdT which was expressed by 13.8% of ALL patients but not in AML patients.

The B-lineage markers that expressed with higher percentage among ALL patients included CD19, CD10 and CD79a. Followed by CD20 and CD22.

Among the T-lineage markers, CD7 was aberrantly expressed in 26.9% of AML patients and CD2 in one AML patient. Only 2 patients with ALL expressed CD7 and cytoplasmic CD3 at the same times.

Cluster of differentiation	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
Myelo-monocytic markers						
CD45	12	46.2	14	48.3	26	47.3
CD13	21	80.8	6	20.7	27	49.1
CD33	20	76.9	6	20.7	26	47.3
cMPO*	11	42.3	2	6.9	13	23.6
CD15	1	3.8	1	3.4	2	3.6
CD14	4	15.4	0	0.0	4	7.3
CD64	9	34.6	0	0.0	9	16.4
CD11c	1	3.8	0	0.0	1	1.8
Aberrant expression						
CD1a	0	0.0	1	3.4	1	1.8
CD4	4	15.4	3	10.3	7	12.7
CD8	1	3.8	2	6.9	3	5.5
CD5	1	3.8	1	3.4	2	3.6

Table 2: *Results of myelo-monocytic and aberrant cluster of differentiation in patients with acute leukemias.*

Leukemia subtype	Provisional diagnosis				Total (n = 55)	
	AML (n = 26)		ALL (n = 29)			
	№	%	№	%	№	%
Acute myeloblastic leukemias						
AML-M1	1	3.8	–	–	1	1.8
AML-M2	6	23.1	–	–	6	10.9
AML-M3	1	3.8	–	–	1	1.8
AML-M4	2	7.7	–	–	2	3.6
AML-M5	3	11.5	–	–	3	5.5
AML (non APL)	9	34.6	3	10.3	12	21.8
Acute lymphoblastic leukemias						
B-ALL	3	11.5	24	82.8	27	49.1
T-ALL	–	–	2	6.9	2	3.6
Mixed phenotype	1	3.8	–	–	1	1.8

Table 3: *Common subtypes of leukemias in the studied patients.*

**c: Cytoplasmic.*

Marker (CD45) was expressed in nearly half of the studied patients with acute leukemias. The myeloid markers that were expressed markedly in AML patients included CD13, CD33 and cytoplasmic MPO. While the monocytic marker that expressed markedly in AML patients was CD64.

Aberrant expression included CD4 in 15.4% of AML patients and 10.3% of ALL patients.

Among the 26 patients provisionally diagnosed as AML, 22 were proved by flow cytometry to be AML subtypes and one was AML with mixed phenotype (biphenotypic). The remainder 3 patients were proved to be B-cell ALL.

The common subtype of AML was non-APL AML. Only one patients was diagnosed as APL.

For the 29 patients provisionally diagnosed as ALL, 26 were proved by flow cytometry to be ALL subtypes and the remainder 3 patients were proved to be AML (non-APL).

The totally diagnosed ALL patients by flow cytometry (n = 29) were B cell type (n = 27) more than T cell type (n = 2).

Discussion

In current period of medicine, when exact diagnosis is needed to manage the patient and also to explain prognosis, immunophenotyping is very useful for acute leukemia. It has diagnostic accuracy of almost 99 %. It can type acute leukemia into AML and ALL and ALL is further subclassified into B-ALL and T-ALL [2].

There are four important methods of diagnosis in acute leukemia i.e. morphology, cytochemistry, cytogenetic and immunophenotyping [3]. Each one has got diagnostic and prognostic importance but obviously immunophenotyping is the best amongst these [4-6].

Markers of progenitors were expressed more in AML than in ALL blast cells, except TdT which was expressed by 13.8% of ALL patients but not in AML patients as mentioned.

The expression of CD34 on a wide range of different cell types, particularly on myeloblasts and in all respects feebly on promyelocytes [7]. CD34 cells can be identified in cord blood, bone marrow and in the peripheral blood of normal subjects, where they establish separately about 1.5% and 0.1 - 0.01% of the components [8]. There is direct relation between the presence of CD 34 expression and poor prognostic value, its absence was associated with a higher percentage of complete remissions [9]. In this study CD34 was positive in 84.6 % in AML patients and 55.2% in ALL patients. Similar result reported by Osman., *et al.* from Sudan wereCD34 reported in 78.7 % in all cases of AML. While in Mansoura study from Egypt CD34 found in 62.1% of non-APL patient, and 76.3% of ALL patients, while in Indian study reported CD34 in 43.9% of ALL patients [10-12].

Positivity of HLA-DR was in 38.5% of AML patients and 34.5% in ALL patients without significant difference between the types of leukemia. This result is parallel to that of an Indian study, while an Egyptian study revealed a high percentage of HLA-DR for both types of leukemias [11,13]. In AML M3 subtype in particular has its own unique immunophenotype which can be differentiated from other FAB subtypes of AML [14]. The combined use of HLA-DR and CD34 was much more helpful in distinguishing cases of non-APL AML from APL cases, than either of these antigens alone. HLADR and CD34 double negativity in APL was ranged to 80% [11,15].

CD117 expression is ordinarily communicated by bone marrow hematopoietic precursors and can be recognizable all through the myeloid lineage until the promyelocyte maturation step and in the erythroid lineage until the professional erythroblast lineage complete maturation of proerythroblast stage [7]. The expression of CD117 in AML was 53.8% and 10.3 % in ALL patient. While in Sudanese study, the percentage of positive expression in AML patients was 83.8%. In Mansoura and Brian studies, the percentages of positive expression were 74.3% and 80% respectively [10,11,16] whereas, in an Indian and Indonesian studies, of ALL patients they were 2.4%, < 5% respectively [12,17].

In our study, TdT was positive and was expressed by 13.8% of ALL patients (Table 1), while it was expressed by 97.4% in Mansoura study (2012) and Bachir., *et al* [11,18].

The B-lineage markers that expressed with higher percentage among ALL patients included CD19 (82.8%), CD10 (75.9%) and CD79a (55.2%). Followed by CD20 (37.9%) and CD22 (27.6%). Similar results were in Bhattacharyya., *et al.* from India CD19 and cytoplasmic CD79a were the most commonly found to be positive in patients with B-ALL, while Subhash., *et al.* CD19 and common ALL antigen CD10 were most common antigen expression [12,19]. In Mansoura study and Bachir., *et al.* all B-ALL cases express CD19 [11,18].

T-lineage-markers

CD7 is the first T-associated antigen to appear during the maturation of T lymphocytes [20]. CD7 and CD3 were common markers, Only 2 patients with ALL expressed CD7 and cytoplasmic CD3 at the same times.CD7 was not totally specific as it was demonstrated to cross react with AML cases [21,22]. Most cases of acute lymphoblastic leukemia of T type express more than one T-lineage marker. Aberrant deletion of one or more pan T-cell antigens is common in T-ALL, however it is a helpful diagnostic finding [23]. All the 2 cases of T-ALL show deletion of one or more of the T-cell antigens used (CD2, CD3, CD4, CD5, CD7 and CD8). Vodinelich., *et al*. (1983), Kaleem., *et al.* (1993) and Traweek., *et al.* (2003) showed that CD7 was the most often expressed T cell antigen [24,25]. While CD5 was the pan-T-cell antigen most often expressed by the T-cell cases in Venkateswaran., *et al* [26].

Cluster of differentiation 45 (CD45) is a protein tyrosine phosphates that is present in all leukocytes with brightest expression on lymphocytes. In addition, it is of prognostic significance as its absence is associated with longer incident free survival in childhood B-cell ALL [27,28]. As well as CD45/side scatter (SSC) gating approach permits efficient discrimination between blasts and normal cells facilitating analysis of blasts present in low proportions [29]. The hematopoietic CD45 marker was expressed in nearly half of the studied patients with acute leukemia. CD45 was expressed in 97.2% of AML cases in Khalidi series [30].

Myelo-monocytic-markers Acute myeloid leukemia was defined immunologically by the expression of 2 or more of the following myeloid markers: myeloperoxidase (MPO), CD13, CD33, and CD117 [31]. Myeloid markers that were expressed markedly in AML patients included CD13, CD33 and cytoplasmic MPO. Same result reported by Al-faleh., *et al.* (2015) from Saudi Arabia [32] while Mansoura study [11] from Egypt and Kaleem., *et al.* [33] reported CD33 followed by CD13 were most common myeloid Antigens. Other study by Byrd., *et al.* [34] showed that CD45, CD33, CD13 were the most commonly expressed Antigens.

The expression of myeloid antigen (CD13) is normally expressed on hematopoietic stem cells, on the immature and mature cells of the myeloid and monocytic lineages, as well as basophiles and eosinophils [35]. Even though, frequently expressed, CD13 cannot be established in all cases of AML, its absence is related to a good prognosis [9]. In this study CD13 is positive in 80.8% of all AML cases. In Mansoura and Bradstock studies, CD13 was (77.9% and71% respectively) which is lower than those in Brian and Ollivier studies (91%, 95%) [11,16,31,36] Cluster of differentiation CD33 is a myeloid antigen and it appears during myeloid differentiation after CD13 at the hemopoietic precursor level [35]. The intensity of expression of CD33 is high on monocytes, and dramatically decreases on basophils, neutrophils and eosinophils [36] CD33 was positive in 76.1% of the AML cases of this study, compared to (91%), (87%) and (79%) in other studies [16,31,36]. The monocytic marker, expressed markedly in AML patients, was CD64 (34.6%) then CD14 (15.4%). Kaleem., *et al.* reported the same results, while Mansoura study reported that CD14was the most common monocytic antigen, followed by CD36 [11,33].

Aberrant Expression: In numerous cases of acute leukemia, blasts of one lineage do not exhibit the markers of normal differentiation but expressed unusual markers in which myeloid associated antigens expressed in lymphoblasts and lymphoid associated antigen expressed in myeloblasts. This phenomenon is called aberrant phenotypes [37,38].

According to our study and other observations, aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia [39-41]. In this study CD7 was aberrantly expressed in (26.9%) of AML patients in agreement with the results of Khurram., *et al.* (2010) and Jahedi., *et al.* (2014) while in difference to the results of El-Sissy., *et al.* (2006) who reported that CD7 was expressed in a minority of his cases [30,42,43]. CD7 expression in AML correlates with a lower incidence of complete remission. Other T lymphoid antigen expressions in AML patients in this study are: CD4 15.4%, CD2 (3.8%), CD8 (3.8%) and CD5 (3.8%). Al-faleh., *et al.* (2015) reported that the most aberrant lymphoid antigens in AML patients were CD2, CD4 and CD7 [32] while Momani., *et al.* study (2016), CD4 was 4.5% [44]. Among B lymphoid antigenic expression, the commonest aberrant marker in AML patients were CD19 followed by CD 20. This results was similar to Momani., *et al.* from Jordon and Sarma., *et al.* (2015) from India [38,44].

In our study the myeloid markers that were aberrantly expressed in ALL patients included CD13 and CD33 with 20.7% which lower than those of AL-Khayed., *et al.* (2015) and Momani., *et al.* from Jordan (47% of CD33 and 37% of CD13), while in Seegmiller., *et al.* (2009) the common aberrant myeloid antigen was CD13, followed by CD33 [44-46].

Conclusion

The incidence of aberrant antigen expression in acute leukemia was comparable with most published international data. In this study CD19 and CD7 were the commonest aberrant lymphoid marker expressed in AML. Further studies are needed to confirm the correlation between aberrant phenotypes with prognosis and therapeutic response of acute leukemia.

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