Asian Hematology Research Journal

2(4): 138- 143, 2019; Article no.AHRJ.53686

Flow Cytometry Findings of Aberrant Expression in a Cohort of Patients with Acute Myeloid Leukaemia

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Authors' contributions

This work was carried out in collaboration among all authors. Author CCK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors BLTB and SACDR managed the analyses of the study, performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Anazoeze Jude Madu, Department of Hematology and Immunology, Faculty of Medical Sciences, University of Nigeria, Nigeria.
Reviewers:
(1) Hosny Badrawy Hamed, Assiut University, Egypt.
(2) Takayuki Takahashi, Japan.
(3) Rocher, Adriana Esther, University Argentina, Argentina.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53686

Short Research Article

Received 10 November 2019
Accepted 14 January 2020
Published 24 January 2020

ABSTRACT

Background: Acute leukaemia is defined as the presence of over 20% of blasts cells in the blood or bone marrow. Acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) are the 2 main types. AMLs have characteristic morphological findings and molecular features with different surface and cytoplasmic cluster of differentiation (CD) markers. These CD markers are determined by immunophenotyping/flow cytometry on leukocytes which helps with accurate diagnosis and reproducibility of AMLs. Flow cytometry plays an important role in the diagnosis, sub classification and monitoring of patients with AML. AML generally shows aberrant CD expression or co-expression in relation to normal myeloid cells.
Objective of the Study: Objective of the Study was to evaluate the frequency and the pattern of aberrant CD expression in AML patients referred to a tertiary care hospital in Sri Lanka in comparison to other published data. There was no comparative data available in respect of Sri Lanka.
Materials and Methods: A retrospective descriptive study including 26 cases of AML diagnosed over a period of 12 months were analyzed. Diagnosis of AML was made by morphology of

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peripheral blood, bone marrow, trephine biopsies, Sudan Black B stain and the immunophenotypic analysis by multiparameter flow cytometry on bone marrow aspirates or peripheral blood. The markers used in flow cytometry were CD 45, CD34, CD19, CD7, smCD3, cyCD3, cyMPO, cyCD79a, CD20, CD15, CD10, CD5, HLADR, CD64, CD13, CD117, CD33, CD14.

Results: Among the 26 AML patients, 15 cases (57.69%) had the conventional CD antigen expressions of myeloid lineage. Other 11 cases (42.3%) were AML with aberrant expression of CD markers. Aberrancies of cyCD3 and CD7 were observed in 54.5% and 45.4% AML cases, respectively. smCD3 in 1 case out of 11 aberrant AML cases. Co expression of T lymphoid markers with myeloid markers occurred in 23% cases in our study. CD13 was not expressed in 1 case out of 5 AML- M4 cases and 1 case out of 7 AML- M1. CD33 was not expressed in 1 case out of 2 AML - M0 cases.

Conclusion: We conclude that aberrant expression of CD markers is seen in a significant population of AMLs. cyCD 3, CD7 and smCD 3 were the aberrant markers present in our study population with cyCD3 showing highest frequency.

Keywords: Leukocytes; flow cytometry findings; aberrant expression; acute myeloid Leukaemia.

1. INTRODUCTION

Acute leukaemia is defined as the presence of over 20% of blasts cells in the blood or bone marrow at diagnosis. Acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) are the 2 main types of acute leukaemias [1].

AML is a cancer of the myeloid lineage of blood cells and it is the commonest form of acute leukaemia in adults with a median age of 65 years. It is diagnosed in (10-15)% of the acute leukaemia in childhood [1].

Flow cytometry plays an important role in the diagnosis, sub classification (Table 1) and monitoring of patients with AML based on the morphological findings. Currently AMLs are classified according to the WHO classification which is based on cytogenetics.

Table 1. French-American-British (FAB) classification of acute myeloid leukaemia

FAB subtype	
AML-M0	AML with minimal differentiated
AML-M1	AML without maturation
AML-M2	AML with maturation
AML-M3	Acute promyelocytic leukaemia
AML-M4	Acute myelomonocytic leukaemia
AML-M5	Acute monoblastic leukaemia
AML-M6	Acute erythroid leukaemia
AML-M7	Acute megakaryoblastic leukaemia

Acute myeloid leukemia generally shows aberrant CD expression or co- expression in relation to normal myeloid cells [2,3]. Several immunophenotypes of blasts cells from AML cases do not show the features of normal CD expression but exhibit the expression of non – lineage specific CD markers. Blasts from AML may lack certain myeloid antigens and possess T- cell and/ or B- cell antigens. This phenomenon is called as 'Aberrant expression' of CD markers in AML. Detection of aberrant CD markers by flow cytometry has been applied toward monitoring of residual disease [4].

2. MATERIALS AND METHODS

2.1 Objective of the Study

The study was done to evaluate the frequency and the pattern of aberrant CD expression in acute myeloid leukemia patients referred to a tertiary care hospital in Sri Lanka in comparison to other published data. No data available in respect of Sri Lanka. AML on therapy, MDS associated AML and those transforming from myeloproliferative disorders were excluded. Those AMLs in which certain markers were not applied were also excluded.

This retrospective descriptive study includes 26 cases of AML diagnosed over a period of 12 months from January 2019 to December 2019.

Diagnosis of acute myeloid leukaemia was done on the basis of morphology of leukaemic cells in the bone marrow, trephine biopsies, peripheral blood smears, results of Sudan Black B stain and the immunophenotypic analysis by multipara-meter flow cytometry for bone marrow aspirates or peripheral blood.

The markers used in the immunophenotypic analysis are the as follows: CD 45, CD34, CD19, CD7, smCD3, cyCD3, cyMPO, cyCD79a, CD20, CD15, CD10, CD5, HLADR, CD64, CD13, CD117, CD33, CD14.

Peripheral blood or bone marrow aspirate was collected in to EDTA anticoagulated tubes. BD FACS Canto TM II Flowcytometer was used and was configured with three lasers to detect up to eight colors. The fluorochromes used were V450, V500c, FITC, PE, PERCP CY5.5, PE CY7, APC, APC H7. The computer workstation was equipped with the calibrated machine with quality control. BD FACS Diva software was used to analyze the results.

The identification of blasts cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters and CD45 intensity versus SSC dot plots. The fluorescence intensities of the blasts were compared with the negative cell population for expression of different CD markers. Expression of a CD marker by more than 20% of the gated population was considered positive [5].

3. RESULTS

Diagnosed AMLs were morphologically classified according to the French-American-British (FAB) Classification (FAB Subtypes) and it is shown in the Table 2 [6].

Though the median age for AML is documented as 65 years in adults, our study showed it was 69.5. Age ranged from 1 to 93 years.

Among the 26 acute myeloid leukaemia patients, only 15 cases (57.2%) had the conventional CD antigen expressions of myeloid lineage – specific markers. Other 11 cases (42.3%) were AML with aberrant expression of CD markers.

cyCD3 showed aberrancy in (54.5%) of aberrant AML cases, CD7 in (45.4%), smCD3 in (9.0%), Co expression of B lymphoid markers with myeloid markers occurred in 0% in our study.

Co expression of T lymphoid markers (CD7, smCD3) with myeloid markers occurred in 23.0% cases in our study. CD13 was not expressed in 1 case out of 5 AML- M4 cases and 1 case out of 7 AML- M1. CD33 was not expressed in 1 case out of 2 AML -M0 cases.

4. DISCUSSION

AML is a clonal proliferation of immature hematopoietic precursors involving primarily in the bone marrow. and blood. Immunopheno-typing by flow cytometry plays an important role in diagnosis of AML.

Side scatter (SSC) and immunophenotyping of blasts helps to differentiate subtypes of AMLs. Aberrant expression of CD markers is important finding in acute myeloid leukaemia which represents a poor prognosis.

There are many studies that have found aberrant lymphoid CD expression in acute myeloid leukaemia. In our study there were 11 cases (42.3%) with such aberrant expression. There are studies with 48%, 58% and 30% reported by Khalidi et al. and John et al., Azad AK et al. and Zhu et al. respectively [7,3].

CD 7 is the commonest aberrant marker found in AML in most studies. In our study the frequency was 45.4% (5 out of the 11 cases). A study by Zheng J et al. revealed that the CD 7 expression was 20.5% [8] with 37% and 24% [9,10] in studies of Bahia DM et al and Reading CL et al respectively. Kita K et al. revealed that young AML male patients with CD 7 expression had a higher incidence of hepatomegaly and central nerve system involvement in contrast to CD7 negative AML patients [11]. It has also been learnt that patients with aberrant expression of CD 7 had responded poorly to the standard chemotherapy with an unfavorable outcome.

Table 2. Number of different AMLs

FAB subtypes	Frequency	Percent	Valid percent	Cumulative percent
AML-M0	2	7.7	7.7	7.7
AML-M1	7	26.9	26.9	34.6
AML-M2	4	15.4	15.4	50.0
AML-M3	7	26.9	26.9	76.9
AML-M4	5	19.2	19.2	96.2
AML-M5	1	3.8	3.8	100.0
Total	26	100.0	100.0	

The aberrant positivity of cyCD3 was the most prominent, 6 out of 11 (54.5%) in our study. Literature survey showed a single study done by Azad A K et al in which the cyCD3 was 8.3% [3].

According to the latest revision (2017) by WHO, T cell component of mixed-phenotype acute leukaemia (MPAL) is characterized by a strong expression of cyCD3, usually with the absence of smCD3 [12] and cyCD3 must be expressed strongly to be considered a T cell specific marker. In our study there was dim expression of cyCD3 in all cases (<25%).

Aberrant expression of B lymphoid markers such as CD 19, cyCD79a and CD20 were negative in our study. But studies have revealed CD 19 and CD 20 in ranges of 10% to 25% of AML cases [13,8]. The study done by Khalidi HS et al. showed CD 20 as the most commonly expressed lymphoid antigen [7].

Table 3. Age and gender distribution of population (Years)

Mean Age	62.88
Median Age	69.5
Minimum Age	1
Maximum Age	93
Female	13
Male	13

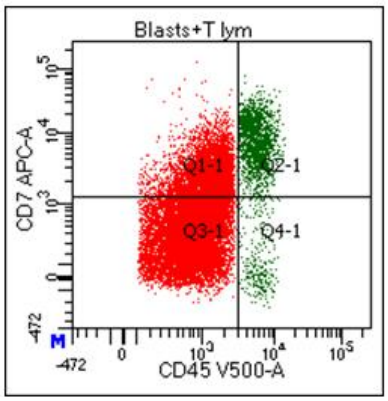

Fig. 1. Aberrant expression of CD7 (32.1%) in blasts (red) in AML- M4

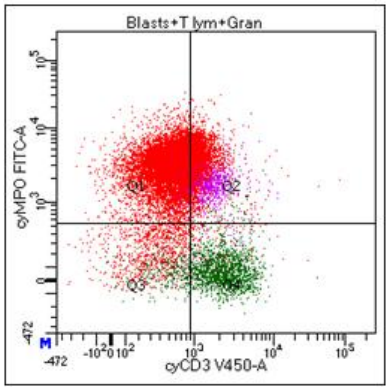

Fig. 2. Aberrant expression of cy CD3 (23.8%) in blasts (red) in AML- M1

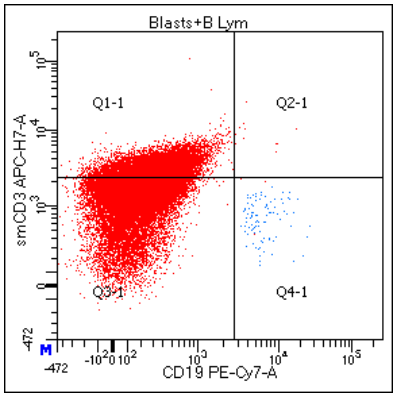

Fig. 3. Aberrant expression of smCD3 (34.1%) in blasts (red) in AML- M2

Table 4. Distribution of acute myeloid leukaemia with aberrant expression

FAB subtypes	Frequency	Percent	Valid percent	Cumulative percent
AML-M0	1	9.1	9.1	9.1
AML-M1	5	45.5	45.5	54.5
AML-M2	1	9.1	9.1	63.6
AML-M4	4	36.4	36.4	100.0
Total	11	100.0	100.0	

Table 5. Individual aberrant markers expression in different AMLs

FAB subtypes	Markers expressed								Pan myeloid markers not expressed	
	cyCD3	cyCD79a	CD19	CD7	smCD3	CD20	CD10	CD5	CD13	CD33
AML-M0	1	-	-	-	-	-	-	-	-	1
AML-M1	2	-	-	3	-	-	-	-	1	-
AML-M2	1	-	-	-	1	-	-	-	-	-
AML-M3	-	-	-	-	-	-	-	-	-	-
AML-M4	2	-	-	2	-	-	-	-	1	-
AML-M5	-	-	-	-	-	-	-	-	-	-
AML-M6	-	-	-	-	-	-	-	-	-	-
AML-M7	-	-	-	-	-	-	-	-	-	-
Total	6	0	0	5	1	0	0	0	2	1

Table 6. Distribution of acute myeloid leukaemia with conventional expression

FAB subtypes	Frequency	Percent	Valid percent	Cumulative percent
AML-M0	1	6.7	6.7	6.7
AML-M1	2	13.3	13.3	20.0
AML-M2	3	20.0	20.0	40.0
AML-M3	7	46.7	46.7	86.7
AML-M4	1	6.7	6.7	93.3
AML-M5	1	6.7	6.7	100.0
Total	15	100.0	100.0	

Table 7. Individual conventional markers expression in different AMLs

FAB subtypes	Markers expressed								
	cyMPO	CD34	CD15	HLA-DR	CD64	CD13	CD117	CD33	CD14
AML-M0	-	2	-	2	-	2	2	1	-
AML-M1	3	6	-	4	-	6	6	7	-
AML-M2	4	3	4	4	1	4	4	4	-
AML-M3	7	1	1	-	-	7	7	7	-
AML-M4	5	2	5	4	4	4	3	5	3
AML-M5	-	-	1	1	1	1	-	1	1
AML-M6	-	-	-	-	-	-	-	-	-
AML-M7	-	-	-	-	-	-	-	-	-
Total	19	14	11	15	6	24	22	25	4

5. CONCLUSION

We conclude that aberrant expression of CD markers is seen in a significant population of AMLs. cyCD 3, CD7 and smCD 3 were the aberrant markers present in our study population. Of these cyCD3 was the commonest.

CONSENT

As per international standard written patient consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53686