

The Role of CD79a in the Diagnosis of Acute Leukemia in Sudanese Patients by Flow Cytometry

MOHAMED GALAL MASOUD YOUNIS¹

Department of Haematology

Royal Care International Hospital, Sudan

ENAAM A. ABDELGADER

Associate Professor of Hematology, Al Neelain University, Sudan

Consultant Clinical Hematologist

Radiation and Isotope Center Khartoum (RICK)

OSAMA A. ALTAYEB

Flow Cytometry Consultant

Flowcytometry Laboratory Khartoum, Sudan

EMAN ABBASS F

Flowcytometry Specialist

Flowcytometry Laboratory, Khartoum, Sudan

AMIN A. AL-AMIN

Clinical Hematopathologist, Flowcytometry Laboratory

Khartoum, Sudan

TARIG M. KARFIS

Medical Laboratory Specialist, Flowcytometry Laboratory

Khartoum, Sudan

MOHAMMED A. MOHAMMED ABDALLA

Consultant Pediatric Oncologist, University of Khartoum, Sudan

Abstract:

Background: Flow Cytometric analysis of leukemia improves accuracy for distinguishing acute leukemia of myeloid and lymphoid origin. In rare cases, both myeloid and lymphoid antigens are expressed, creating ambiguity for lineage assignment and difficulties in diagnosis of leukemia. CD79a is a highly lineage-specific marker of B lymphoid cells and plays an important role in the diagnosis of acute leukemia, The lineage of the blast cells is defined by microscopic

¹ Corresponding author: mohamed15021@hotmail.com

examination (morphology), immunophenotypic (flow cytometry), cytogenetic and molecular analysis.

Aim: The aim of this study is to confirm that the expression of CD79a is specific marker for the diagnosis of B-ALL, and can be aberrantly expressed in another types of acute leukemia.

Methods: fifty three patients (53) who were newly diagnosed as acute leukemia were included in this study and immunophenotyping with flowcytometry was done using the international guidelines for CD markers, and the role of CD79a was studied.

Result: We found that CD 79a can be expressed in all types of acute leukemia but it is very specific for B-cell ALL as there is a highly significant difference between B-ALL and other types of acute leukemia with CD 79a expression (p-value 0.000).

Conclusion: CD79a is very specific for B-cell ALL, however it can be aberrantly expressed in other types of acute leukemia.

Key words: Sudan Black B, B-ALL, AML, Biphenotypic, flow cytometry.

Introduction:

Acute leukemia is normally defined as the presence of 20% or more of blast cells in the bone marrow at clinical presentation. However, it can be diagnosed with less than 20% blasts if specific leukaemia - associated cytogenetic or molecular genetic abnormalities are present. ⁽¹⁾ .This clonal evolution model of cancer development involves gain of function of oncogenes and loss of function of tumor suppressor genes that cooperate to induce fulminant disease. ⁽²⁾ Acute leukemia can be classified into two main groups: acute lymphoblastic leukaemia (ALL) and acute myeloid (myeloblastic) leukaemia (AML). Rare cases are undifferentiated or mixed. Sub classification of ALL or AML depends on morphological, immunological, cytogenetic and molecular criteria. ⁽³⁾ ALL sub classified into two main group B cell-ALL and T cell-ALL. B-cell ALL defined as an aggressive

tumor of immature B-cell precursors always positive for cytoplasmic CD79a as well as B-cell prolymphocytic leukemia are positive for CD79a. Rare cases of acute myeloid leukemia AML with recurrent genetic aberrations AML with t (8; 21) (q22; q22); are positive for cytoplasmic CD79a. While acute myeloid leukemia not otherwise specified and AML without maturation are negative. ⁽⁴⁾

All hematopoietic progeny are derived from pluripotent stem cells as it mature, they lose stem cell markers and acquire lineage-specific antigens the surface and cytoplasmic markers expressed in hematologic malignancies resemble those of normal hematopoietic cell differentiation. Frequently, neoplastic cells arrest at a particular stage of development and display aberrant antigenic patterns. Flow cytometry was originally designed to measure physical properties of cells based on their ability to deflect light. Over the years, it has evolved to include detection of fluorescent signals emitted by dyes bound directly to specific molecules or attached to proteins through monoclonal antibodies. ⁽⁵⁾ CD79a is a product of the human mb-1 gene. Together with CD79b is a B-cell receptor (BCR) form a disulfide-linked heterodimer, which is non-covalently associated with the membrane immunoglobulin on B cells located on chromosome 19q13.2 have a molecular mass of 33 (KD) restricted to B lymphocytes, first appear on the surface of pro-B cells and remaining throughout the stages of B-cell differentiation, except in plasma are important during B-cell development and B-cell function.⁽⁶⁾ The immunological characterization of leukemic blast cells in terms of their lineage commitment and differentiation stage has so far mainly been based on the analysis of surface marker molecules. Mostly for technical reasons, and this is particularly true for flow Cytometric analyses, most investigators avoided intracellular markers in their evaluation panel and restricted their studies to easily accessible surface antigens. During the last several

years it became evident, however, that the most reliable lineage markers, already expressed very early in differentiation, are in fact localized within the cytoplasm. ⁽⁷⁾

Raymond Lai et al found that all cases of acute myeloid leukemia were CD79a-, whereas all cases of B-lineage acute lymphoblastic leukemia (ALL) were CD79a+. Three of 8 cases of T-cell ALL showed variable CD79a expression, indicating the presence of a blast subset expressing a relatively high level of CD79a. ⁽⁸⁾Kozlov et al cited that in 89 cases of AML, 2 showed strong expression of CD79a. Both cases were differentiated FAB AML-M2 and demonstrated the t (8; 21) with cytogenetic and the AML1/ETO rearrangement with fluorescence in situ hybridization (FISH). The immunophenotyping met proposed scoring criteria for a diagnosis of B-ALL. Nevertheless, the Cytogenetic and FISH findings indicate that CD79a, despite its specificity for B-cell differentiation, represented the aberrant presence of a B-cell antigen in leukemia's of distinct myeloid lineage. It is doubtful that, in this setting, CD79a expression should be considered a manifestation of lineage ambiguity. ⁽⁹⁾Cruse JM et al found that Among 46 patients with AML four expressed CD79a. CD79a is a B cell marker that is assigned a high score of 2.0 in the differentiation of acute leukemia's of ambiguous lineage in the WHO classification. The aberrant expression of CD79a, representing the capacity of these leukemia for trilineal expression of leukocyte differentiation antigens, portends a poor prognosis.⁽¹⁰⁾ In other study done by Ghaleb Elyamany et al reported that an unusual case of AML in which CD79a is clearly expressed along with other lymphoid antigens (CD7 and CD56).⁽¹¹⁾

The aim:

The aim of this study is to confirm that expression of CD79a is a specific marker for diagnosis of B-ALL, and apparently expressed in another types of acute leukemia.

Materials and methods:

A total of 53 acute leukemia (AL) cases immunophenotyped at flowcytometry laboratory, Khartoum, using 4 colours flow cytometer were reviewed. The analyzed samples were either of peripheral blood (PB) or bone marrow aspirate (BMA) according to availability and presence of blast cells. Morphological examinations to all films was first done followed by cytochemical stains (Sudan Black B).

Study design:

This is a descriptive cross-sectional study consisted of 53 patients newly diagnosed as acute leukemia their ages between (1-77) years old, from both genders conducted during March 2015 at flow cytometry Laboratory, Khartoum-Sudan

Sampling:

Representative fresh samples (whole blood or BM) taken in EDTA.

Quality Control:

Depending up on pilot study in the quality control results (that saved in the Q.C system II software file) of EPICS XL flow cytometer, which adjusted the cut off points between negative and positive scale for every marker, Positivity was considered when $\geq 20\%$ of the population expressed the marker. The parentheses was also recorded for most of the markers.

Data Analysis:

Statistical analysis was done using SPSS 17, correlation tests were done using Pearson correlation coefficient.

Methodology:

50 µL of the cell suspension (whole blood, bone marrow, or mononuclear cells) transferred to a test tube 100 µL of fixation reagent was added and mixed gently then incubated at RT for 15 minutes after that 2 ml PBS were added and mixed gently by using a vortex mixer then centrifuged at 540g for 5 minutes. The supernatant aspirated, leaving 50 µL of fluid. 100 µL of permeabilization reagent mixed and added. 10 µL of cyt-79a-PE was added. Mixed gently then incubated in the dark place at RT for 15 minutes. 2 ml PBS were added and mixed gently by using a vortex mixer. Centrifuged at 540g for 5 minutes. The supernatant aspirated, leaving 50 µL of fluid. The cell pellet Resuspend in 300 µL PBS and acquire on a flow cytometer within the first three hours of finishing the sample preparation.

Results:

The age group (5-12 years) is 40%. (13-45 years) and (46-70 years) had the same percentage 30%. (Fig 2) Female is 57%, Male is 43 %,bone marrow sample is 58%, peripheral blood is 42% , ALL is 62%, AML is 32%, biphenotypic AL is 6%. (Fig 3) subclass of acute leukemia B-ALL is 58% AML M4 is 9%, M5, M2 &biphenotypic is 6%, M0 is 5%, M3 &T-ALL is 4%, M1 is 2%, ,(Fig 5). AML negative is 94%, weak positive is 6%, (Fig 5). ALL moderate positive is 49%, strong positive is 30%, weak positive is 15%, negative is 6 %.(Fig 5) biphenotypic AL moderate positive is 67% weak positive is 33 %.(Fig 5). According to percent degree with subclass of acute leukemia, all AML subclass is (100%) negative for CD79a including (M0 M1 M2 M3 M), plus M4 which show 20% of positivity. In T-ALL cases weak and moderate expression of marker is equal (50%). In B-ALL all degree is noted from negative weak moderate to strong expression (6%, 13%.48%, 32%) respectively. in

biphenotypic AL moderate constitute (67%) of cases while weak is (33%) (Fig 6)

Fig (1) CD79a expression in B-ALL

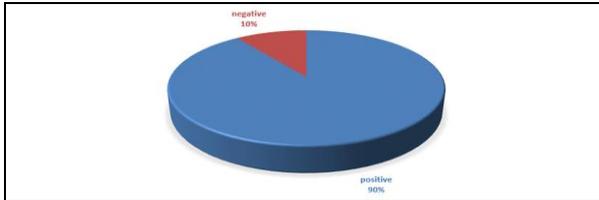


Fig (2) Age group

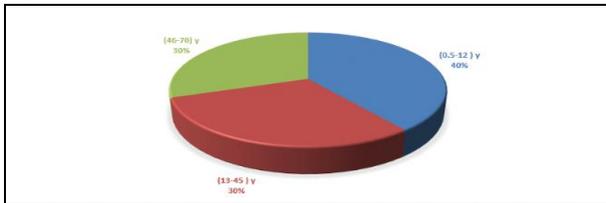


Fig (3) Types of acute leukemia

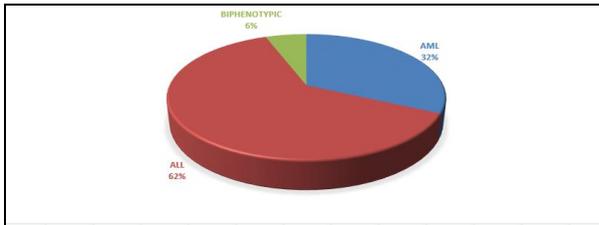


Fig (4) subclass of acute leukemia

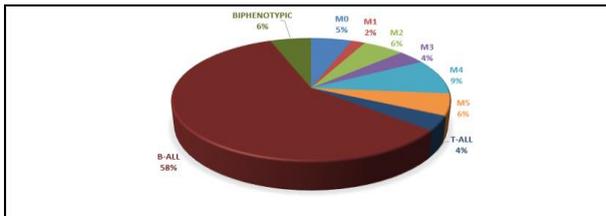


Fig (5) CD79a expression

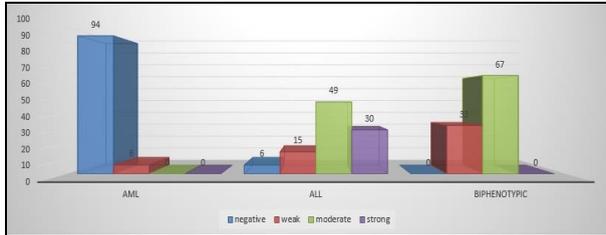


Fig (6) CD79a percentage.

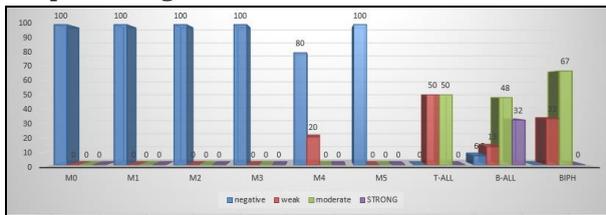
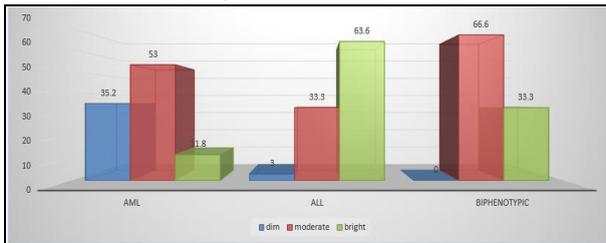


Fig (7) CD79a mean intensity



Discussion:

Of the 53 case of acute leukemia studied 31 cases are B-ALL 28 cases (90 %) of them were positive for CD79a while 3 cases (10%) were negative (Fig 1). Our study showed that CD79a is very specific to identification and differentiation of B-ALL from other types of acute leukemia, there is highly significant difference between B-ALL and other type of acute leukemia with CD79a expression (p-value 0.000). (Fig 5) Most cases of B-ALL showed bright expression of CD79a (P value=.002). (Fig 7).

This finding agree with Verschuren MC in that mb-1 molecule as a pan-B-cell marker for the diagnosis of immature and mature B-cell malignancies. The expression pattern of the mb-1 protein is comparable to that of the CD19 and CD22 antigens, but has the advantage of being B-lineage specific.¹²

According to percent degree and mean intensity, the marker didn't helpful in differentiation between B-ALL subclasses since it had the same expression among B-ALL subclasses. (P value .061) (P value 0.116) respectively.

Two cases of T-ALL were examined they showed positive result with weak to moderate mean intensity expression, which confirm that subset of T-ALL blasts have aberrantly expressed high mean intensity of CD79a expression which agree with Raymond Lai et al finding.⁹ we need more sample of T-ALL to study this marker expression in T-ALL.

AML have moderate CD79a expression (p. value .002) CD79a was aberrantly expressed in AML M4, Cruse JM et al cited that no CD79a expression in the M4 cases which disagree with our finding.¹¹ Ghaleb Elyamany found that aberrantly these markers were expressed early in hematopoietic ontogeny in the lesser-differentiated acute myeloid leukemia subtypes, including FAB M0, M1, and M2. We did not find any expression of CD79a in M0 cases, this finding was disagree with Ghaleb Elyamany findings.

Conclusion:

CD79a is playing a crucial role in the identification and differentiation of all B-ALL cases due to high specificity. Put in consideration the presence of CD79a in some cases of AML.

Recommendations:

Further study with big number of cases is needed.

REFERENCES:

1. A. V. Hoffbrand. A. H. Moss (2011), *Essential Haematology* (6th.Ed.) Blackwell Publishing p.179-1 ISBN 978-1-4051-9890-5
2. John P. Greer MD (2103), *Wintrobe's Clinical Hematology*(13th.Ed.) p. 3479-1 ISBN-13: 978-1451172683
3. Atul B. Mehta A. Victor Hoffbrand (2014), *Haematology at a Glance* (4th. Ed.) p.66-1 ISBN-13: 978-1119969228 John Wiley & Sons, Ltd
4. Aster, Jon C. (2013), *High Yield Pathology Hematopathology. p.92-1* –ISBN: 978-1-4377-1758-7
5. Bernadette F. Rodak (2012), *Hematology Clinical Principles and Applications* (4th Ed). p. 490-495 ISBN: 978-1-4377-0692-5
6. John P. Greer MD (2009). *Wintrobe's Clinical Hematology. (12th. Ed.)* p. 2544-1 - ISBN 978-0-7817-6507-7
7. Knapp W1, Strobl H, Majdic O. Flow cytometric analysis of cell-surface and intracellular antigens in leukemia diagnosis. *Cytometry*. 1994 Dec 15; 18(4):187-98.
8. Lai R1, Juco J, Lee SF, Nahirniak S, Etches WS. Flow cytometric detection of CD79a expression in T-cell acute lymphoblastic leukemias. *Am J Clin Pathol*. 2000 Jun; 113(6):823-30.
9. Kozlov I, Beason K, Yu C and Hughson M. CD79a expression in acute myeloid leukemia t(8;21) and the importance of cytogenetics in the diagnosis of leukemias with immunophenotypic ambiguity. *Cancer Genet Cytogenet*. 2005; 163:62-7.
10. Cruse JM, Lewis RE, Pierce S, Lam J and Tadros Y. Aberrant expression of CD7, CD56, and CD79a antigens

- in acute myeloid leukemias. *Exp Mol Pathol.* 2005; 79:39-41.
11. Elyamany G, Fadalla k and Abdulaaly AA. Abnormal expression of CD79a, CD56 and CD7 in acute myeloid leukemia. *Pathol Discov.* 2013; 1:6.
 12. Verschuren MC1, Comans-Bitter WM, Kapteijn CA, Mason DY, Brouns GS, Borst J, Drexler HG, van Dongen JJ. Transcription and protein expression of mb-1 and B29 genes in human hematopoietic malignancies and cell lines. *Leukemia.* 1993 Dec; 7(12):1939-47.