CD117 IN DIFFERENTIATION ACUTE MYELOID LEUKEMIA AND ITS CO-EXPRESSION IN ACUTE LYMPHOBLASTIC LEUKEMIA BY FLOW CYTOMETRY IN SUDANESE PATIENTS

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ABSTRACT

The CD117 is a specific marker for diagnosis of acute myeloid leukemia (AML), also expressed in some acute lymphoblastic leukemia (ALL) cases. The aim of this study to classify AML and to differentiate the co-expression of CD117 in ALL by flow cytometry which used to detect the positive rate and expression level of CD117 in bone marrow mononuclear cells (BMMNCs), and peripheral blood (P.B). The results of the study showed that CD117 expression in 62% of AML while 00% in ALL. In addition, in AML sub group revealed that, M0 77%, M2 59%, M3 46%, M4 56%, M5 11%.M 61%, M7 10%. The study of mean intensity expression of CD117 among AML sub group (M0, M2, M3, M4, M6, and M7) were moderate expression (60%, 50%, 100%, 71.4%, 100%, and 100%) respectively, while (M5) were strong dim expression (100%). The expression of CD117 peak width among AML sub group the result showed that there was (M0) were heterogeneous and (M2, M3, M4, M5, M6, M7) were homogenous. The study concluded that CD117 expression is a specific and rather sensitive marker for differential diagnosis between AML and ALL.

KEYWARDS: Acute Myeloid Leukemia, CD117, co-expression, Acute Lymphoblastic Leukemia.

1. INTRODUCTION

Acute leukemia are a heterogeneous group of malignancies with different clinical, morphologic, immunologic, and molecular characteristics,[1,2] and highly proliferated in blood stream, that characteristically comes on suddenly and, if not treated, progresses quickly. In acute leukemia, the leukemic cells are not able to mature properly. There are two types of acute leukemia acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL).[3]

1.1 Acute Myeloid leukemia: The Acute Myeloid Leukemia (AML) is about 20% of acute leukemia in children and 80% of acute leukemia in adults. Immunophenotyping has become very important not only in diagnosis and sub classification of AML but also in the detection of the minimal residual disease.

Acute myeloid leukemia (AML) is a type of cancer that affects the blood and bone marrow. It is a complicated, heterogeneous disease including the presence of a clonal expansion of neoplastic myeloid cells with variable degrees of differentiation and different clinical, morphologic, immunologic, and molecular characteristics.[4,5,6]

AML classified into sub-group by certain system including FRENCH AMERICAN BRITISH (FAB), WHORLD HEALTH ORGANIZATION (WHO) and Immunophenotyping by flow cytometry in which cell surface antigen expression.[7]

1.2 FAB classification: Recommendations for classification depend on morphology and Cyto chemical staining. The FAB classification now has been extensively distributed for studying AML by many researchers all over the world.[8, 9, 10]

1.3 Subtypes include
- Acute myeloblastic leukemia, minimally differentiated (M0)
- Acute myeloblastic leukemia without maturation (M1)
- Acute myeloblastic leukemia with maturation (M2)
- Acute myelomonocytic leukemia (M4)
- Acute monoblastic leukemia (M5a) and acute monocytic leukemia
- Acute erythroid leukemia (M6)

- Acute megakaryoblastic leukemia (M7)

1.4 Acute lymphoblastic leukemia: Acute lymphoblastic leukemia (ALL): is the most common pediatric cancer; it also strikes adults of all ages. Malignant transformation and uncontrolled proliferation of an abnormally differentiated, long-lived hematopoietic progenitor cell results in a high circulating number of blasts, replacement of normal marrow by malignant cells, and the potential for leukemic infiltration of the CNS and abdominal organs.[11]

FAB classification of acute lymphoblastic leukemia (for historical purposes)
ALL-L1: Small cells with homogeneous nuclear chromatin, a regular nuclear shape, small or no nucleoli, scanty cytoplasm, and mild to moderate basophilic.

ALL-L2: Large, heterogeneous cells with variable nuclear chromatin, an irregular nuclear shape, or more nucleoli, a variable amount of cytoplasm, and variable basophilia.[12]

ALL-L3: Large, homogeneous cells with fine, stippled chromatin; regular nuclei prominent nucleoli; and abundant, deeply basophilic cytoplasm. The most distinguishing feature is prominent cytoplasmic Vacuolation.[13]

Early classification systems for acute leukemias were based only on cytomorphological and cytochemical investigations. Morphology still plays a central role, but current classification systems have incorporated immunophenotyping in order to achieve greater precision in delineating the hematopoietic lineage and differentiation stage of particular leukemias.[14]

Immunophenotyping is fundamental for classifying lymphoid malignancies and is also essential for recognizing several subtypes of acute myeloid leukemia (e.g. AML-M0 and AML-M7) and biphenotypic acute leukemias; for monitoring the responses to treatment, including detection of minimal residual disease (MRD); and for identifying markers with prognostic implica-tion.[15] Immunophenotyping has become extremely important not only in diagnosis and sub classification of AML but also in the detection of the minimal residual disease. It is also suggested to have prognostic significance.[16]

2. MATERIAL AND METHODS
A total of 68 (48 AML, 16 ALL) acute myeloid leukemia (AML) cases newly diagnosed, adult and pediatric, both genders at Radioisotope Centre Khartoum (RICK). Then they immunophenotyped at flowcytometry, of Dr. Osama laboratory.

2.1 Sample Collection and Preparation
Venous blood sample: 2.5 ml of venous blood sample was collected in EDTA vacotainer (5ml). Bone marrow

aspiration: 2 ml of bone marrow aspiration was collected in Lithium Heparin vacotainer (5ml).

2.2 Lysing procedure for whole blood and B.M aspiration monoclonal antibody combination
1. The tubes were labelled for analysis.
2. 20 uL of monoclonal antibody was added into each tube.
3. 100 uL of sample was added containing no more than 1 x 10 leukocytes / ml. (Counted by hematology analyzer - SYSMEX).
4. Each tube was vortexes for 5 seconds.
5. Each tube was incubated at room temperature (18-25 C) for 15 minutes.
6. Add 1 ml of the "fix-and-lyse" mixture was added to the tube and vortexes immediately for three seconds.
7. Each tube was incubated at room temperature for at least 10 minutes and was protected from light.
8. Centrifuge the tube at 150 x g for 5 minutes and discard the supernatant by aspiration.
9. 3 mL of PBS was added.
10. All tubes were centrifuged in 150 x g for 5 minutes and the supernatant were discarded by aspiration.
11. The pellets were Re-suspended by addition of 0.5 to 1 mL of 0.1% formaldehyde.
12. All tubes were vortexes for 5 seconds.
13. All tubes were analyzed by the flow cytometer.

2.3 Flowcytometric immunophenotyping
Samples were stained by monoclonal antibodies for the CD117 ((Immunostep-Spain). After that the percentage group of the marker was detected by flow cytometer (COULTER® EPICS® XL™ Flow Cytometer).

2.4 Ethical consideration: The aims methods of this study was fully explain to the patients and their consent to participate in this study were obtain. Sample was taken form patient who consent to participate. The questionnaire was full in the presence of patient.

3. Statistical Analysis
Statistical Package for Social Sciences (version 13) was used for analysis and to perform Pearson Chi-square test for statistical significance and one way an ova for numerical data.

Determination of remark result for percentage group: depending up on pilot studying 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 of scoring markers, the remark results were determined as follow: (Table 1).

Table. (1): show the CD117 percentage group.

Group	Result	Interpretation
1	31-50	Weak
2	51-70	Moderate
3	71-100	Strong

Determination of remark result for mean intensity group: 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 of scoring markers, the remark results were determined as follow: (Table 2).

Table. (2): show the CD117 mean intensity group.

Mean Group	Result	Interpretation
1	0.0-1	Dim
2	1.1-4	Moderate
3	4.1-17	Bright

Determination of remark result for peak width group: 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 of scoring markers, the remark results were determined as follow: (Table 3).

Table. (3): Show the CD117 peak width group.

Peak Width Group	Result	Interpretation
1	1-22	Homogenous
2	23-44	Heterogeneous

4. RESULTS

The study conducted 64 patients including 48AML 16 ALL and the samples collected from B.M. (62.5%) and sample collected from P.B. (37.5%). The results showed that the expression of CD117 among AML sub group cases (M0, M1, M2, M3, M4, M5, M6, and M7), was (7.8%, 0%, 1.65, 18.85, 14.1%, 4.75%, 1.6%, and 4.7%) respectively (Figure1). Moreover, the expression of CD117 remark distribution showed 62% in AML without expression of CD117 for ALL (Figure2). In addition, the result of CD117 remark positive among AML sub group showed that (M0, M2, M3, M4, M5, M6, M7,) was (100%, 66.7%, 58.3%, 77.8%, 33.3%, 0%, and 0%) respectively as shown in figure (3). The flow cytometry has classified the 48 patient of AML into three categories: percentage, mean intensity and peak width. The results of CD117 percentage showed the expression on AML sub-class M0, M2, M3, M5, M6, M7, were (77%, 59%, 46%, 56%, 11%, 1%, 10%), respectively (Figure 4). Among the expression of CD117 percentage AML sub classification the result also showed that most (M0, M2, M3) was strong positive (80%, 80%, 71.4%) respectively, some of (M4) moderate positively (57.1) and most (M5) were weak positive(100%) (Figure 5). The study of CD117 mean intensity expression among AML sub group (M0, M2, M3, M4, M6, M7) the result was moderate expression (60%, 50%, 100%, 71.4%, 100%, 100%) respectively, while (M5) was strong dim expression (100%) (Figure 6), therefore CD117 mean intensity group had no significant role in AML sub group except (M5) by expression of CD117 peak width among AML sub group the result show that there was (M0) were heterogeneous and (M2, M3, M4, M5, M6, M7) were homogenous as shown in figure (7).

Table (4) Shows the proprieties of CD117 among the sample population Sub group M0 show S\M for CD117 percentage, +ve for CD117 remark, M\B for CD117 min intensity and heterogonous for CD117 peak width, Sub group M2 show S\rare M\W for CD117 percentage, +ve\-ve for CD117 remark, M\B rare D forCD117 min intensity and heterogonous\ homogenous for CD117 peak width, Subgroup M3 show S\M for CD117 percentage, +ve\-ve for CD117 remark, M for CD117min intensity and heterogonous\ homogenous for CD117 peak width Sub group M4 show S\M rare W for CD117.

Table. 4. Show CD117 proportion among acute leukemia.

Sub group of AML	CD 117 %	Cd117 remark	CD 117 min	CD 117 pw
M0	S\M	+ve	M\D,B	Hetro
M2	S\rare M \W	+ve\-ve	M\B rare D	Hetro \Hemo
M3	S\M	+ve\-ve	M	Hetro \Hemo
M4	S\M rare W	+ve\-ve	M rare D,B	Hetro \Hemo
M5	W	-ve\+ve	D	Hetro \Hemo
M6	—	-ve	M	Hemo
M7	—	-ve	M	Hemo

Key words: S:Strong,M:Moderate,W:Weak,D:Dim,B:Bright,Hetro:heterogynous, Homogenous,+ve: Positive,-ve: Negative and \:or.
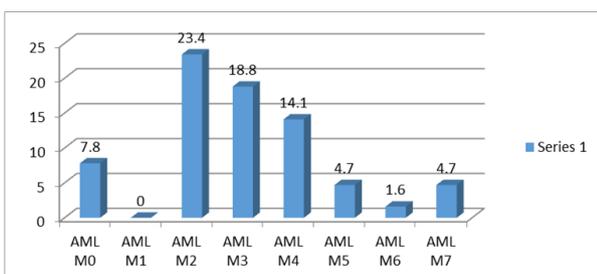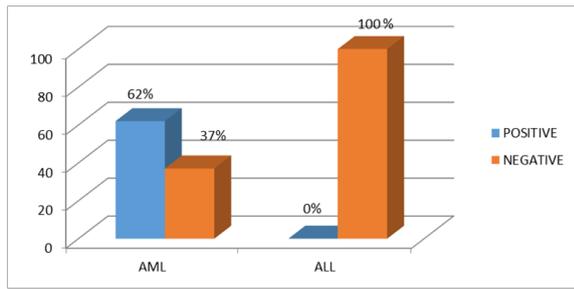
Figure. 1. Show AML distribution.

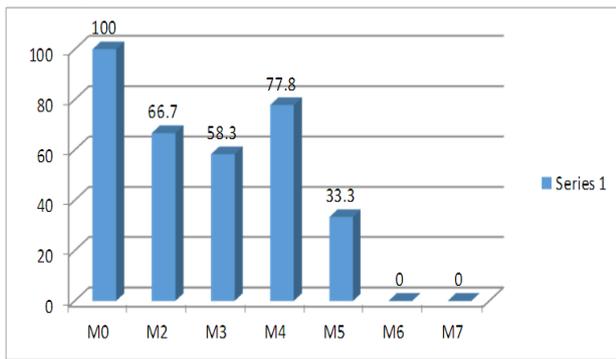**Figure. 2. Show CD117 remark distribution.**

Figure. 3. Show CD117 positive AML sub group distribution.
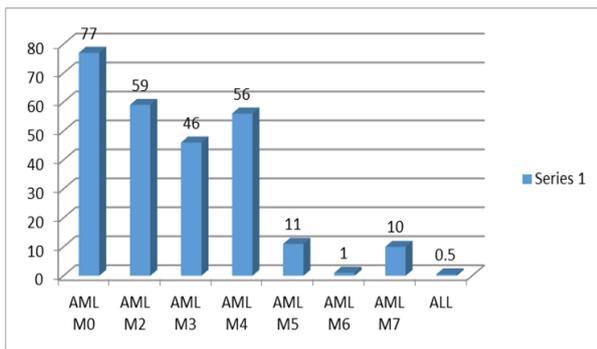

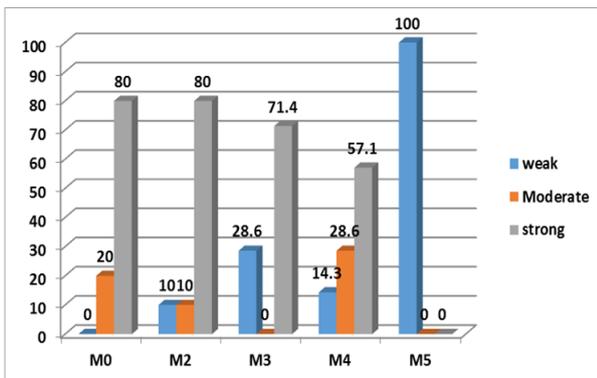**Figure. 4. Show CD117 mean percentage among AML sub group.**

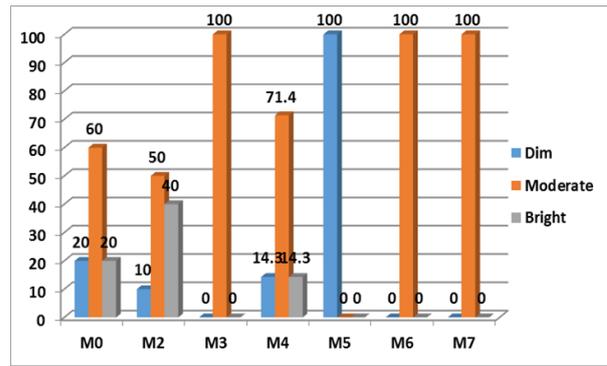**Figure. 5. Show CD117 percentage group.**

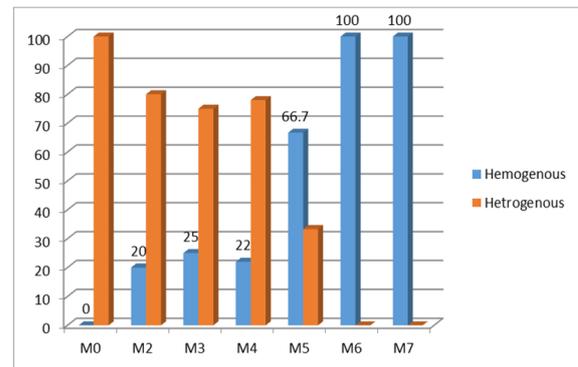**Figure. 6. Show CD117 mean intensity group.**

Figure. 7. Show CD117 peak width group.

5. DISCUSSION

CD117 is a specific marker for AML, we found CD117expression in 62% of AML others have reported CD117 expression by flow cytometric analysis in 23%-91% of AML cases .Our finding are in agreement with the study of Abbas Ahmadi, Ali –Akbar, et.al, tumor biology.04/2014; 35(7). study the Diagnostic value of CD117 in differential diagnosis of acute leukemia,[17] they concluded that, CD117 expression is a specific and rather sensitive marker for differential diagnosis between AML and ALL.

When we study CD117 mean percentage among AML sub group we found significant difference b\w them, M0, M2, M3, M5, M6, M7, were (77%, 59%, 46, 56%11%, 1%, 10 %), of AML cases respectively. Our finding also agreement with the study of Cascavilla N, Musto p, et al. CD117 (c-kit) is a restricted antigen of acute myeloid leukemia and characterized early differentiative levels of M5 FAB subtype,[18] they conclude that the CD117 antigen shows a high specificity for AML independently upon FAB classification, and represents a reliable marker in characterizing the differentiate degree of the monocytic blasts,James O. Newell, Melissa H.Cessna, etal, 2003 Mar; 52(1):40-3., Salt Lake City, Utah, USA. Importance of CD117 in the evaluation of acute leukemia's by flow cytometry, this study highlights the importance of CD117 as specific marker need to use in determined of AML, and in sometimes With AML (M2) sub types with an8:21 translocation, where leukemic blast did not show expression of myeloid markers cd13

or cd33, and Christine P. Hans, MD, William G. Finn, MD, et al, 2002; 117:301-305 Abstract quote. We assessed that the diagnostic usefulness of adding anti-CD117 to our existing flow cytometric profile, CD117 is a specific marker for myeloblastic leukemia's. Sensitivity is greatest in French-American-British M2 and relapsed AML.

6. CONCLUSION AND RECOMMENDATION

Conclusion: We conclude that the CD117 antigen shows significant diagnosed against acute leukemia (AML/ALL), and in diagnosed of acute myeloid sub classification.

Recommendation

We recommended that using of flow cytometry in the diagnosis of acute leukemia. Moreover, using the CD117 routinely in the differentiation b/w AML/ALL, and identification of AML sub group instead of panel cells to minimized cost.

7. ACKNOWLEDGMENT

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