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Study of Myeloid Antigens CD13 and CD33 Expression in Sudanese Patients with Acute Leukemia

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Abstract:

Background: *Acute leukemia is a heterogeneous group of malignancies which are varying according to their clinical, morphologic, immunologic, and molecular features and display different characteristic patterns of surface antigen expression. This*

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study was conducted to study the myeloid antigens CD13 & CD33 expression in Sudanese patients with acute leukemia attended the Flowcytometry Laboratory in Khartoum, Sudan.

Materials and Methods: *It was a descriptive cross-sectional study. Flowcytometry was used for immunophenotyping of acute leukemia using bone marrow or peripheral blood samples for the analysis. All samples prepared by incubation with anti-CD13 and anti-CD33 antibodies (Immunostep, Salamanca, Spain) for 10 minutes in dark place then added 1 ml of RBC lysis solution and run by the flowcytometer (Epics XL-MCL, Beckman Coulter, Miami). The flowcytometry parameters were calculated by SYSTEM II software and all the significant p.values were calculated by SPSS program.*

Results: *The study included 50 Sudanese patients with acute leukemia: Of these 29 cases were of Acute Myeloid Leukemia (AML), 19 of Acute Lymphocytic Leukemia(ALL), one case of Biphenotypic Acute Leukemia (BPHAL) and one case of Undifferentiated Acute Leukemia(UNDIFF)(Table 1). CD13 remark in (AML) was 65.5% positive, (ALL) was 100% negative, (BPHAL) was 100% positive, and in (UNDIFF) was 100% positive (p.value = 0.000) (Figure. 2). CD33 remark in (AML) was 79% positive, (ALL) was 26% positive, (BPHAL) was 100% positive and in (UNDIFF) was 100% negative (p.value= 0.000)(Figure 6).CD13 in AML sub-group showed that CD13 in M0 was (66%) positive, M1 was (100%) positive, M2 was (57%) positive, M3 was (25%) positive, M4 was (100%) positive, M5 was (100%) positive, M6 was (100%) negative and M7 was (100%) negative. CD33 in AML sub groups showed that M0 was (33%) positive, M1 was (100%) positive, M2 was (85%) positive, M3 was (75%) positive, M4 was (60%) positive, M5 was (100%) positive, M6 was (100%) positive and M7 was (100%) positive (Figure 8). CD13 remarks in ALL sub groups showed that (100%) of T-ALL and B-ALL were negative. CD33 remark showed that (100%) of T-ALL was negative and (33%) of B-ALL were positive considered as aberrant expression (Figure 9). Flowcytometry patterns of CD13 and CD33 antigen expression showed that all of the remark results, marker percentage and mean fluorescence intensity were highly significant in the differentiations between AML and ALL cases which mean that we can use CD13 and/or CD33 in the identification and diagnosis of AML cases and sometimes in the exclusion of some ALL cases(in which CD33 present*

as aberrant expression)with putting in the consideration the few cases of B-ALL which showed some CD33 aberrant expression.

Conclusion: *We conclude that CD13 and CD33 are specific markers for myeloid lineage with some aberrant expression in other types of acute leukemia among the Sudanese population. The presence of aberrant expression helps to identify a neoplastic process, accurate diagnosis and follow up.*

Key words: AML, B-ALL, T-ALL, flow cytometry.

Introduction:

Acute leukemiaare a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood. These abnormal cells cause symptoms of: (i) bone marrow failure (i.e. anemia, neutropenia, and thrombocytopenia) and (ii) infiltration of organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin or testes). [(1)] The acute leukemia is broadly classified as acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). The blast cells show varying clinical, morphologic, immunologic, and molecular features and display characteristic patterns of surface antigen expression (CD antigens).[(2)] Distinction between lymphoid and myeloid leukemia, most often made by flowcytometry, is crucially important.

(AML) is characterized by an increase in the number of immature cells in the bone marrow and arrest in their maturation. This condition frequently results in hematopoietic insufficiency with or without leukocytosis. [(3)] AML is the most common acute leukemia affecting adults, and its incidence increases with age. [(4)] AML has several subtypes; treatment and prognosis varies among subtypes. Five-year survival varies from 15–70%, and relapse rate varies from 33–78%, depending on subtype.[(5)] (ALL) is biologically and clinically a

heterogeneous group of diseases characterized by malignant proliferation and accumulation of immature lymphoid cells within the bone marrow, blood, and lymphoid organs.(6) The proliferating lymphoid progenitor cells, arrested in various stages of maturation, can be identified as B-, T-, or mixed-lineage leukemia.(7) ALL is most common in childhood with a peak incidence at 2–5 years of age, and another peak in old age. Cure is a realistic goal, as ≥94% of children and 30–40% of adults have continuous disease-free survival for five years. (8)FAB classification is based on the morphological characteristics of Wright-stained cells in peripheral blood or bone marrow and cyto-chemical staining of blasts. The FAB system groups AMLs into subtypes (M0 through M7) and ALL into three categories (L1 through L3). This system is based on the type of cell from which the leukemia developed and the maturity of cells. Classifications are based largely on how the leukemia cells look under the microscope after routine staining. In a revision of the original criteria, more than 30% blasts in the marrow suffice for the diagnosis of acute leukemia in any of the categories. The World Health Organization (WHO) classification uses all available information— morphology, cyto-chemistry, immunophenotype, genetics, and clinical features—to define clinically significant disease entities. (9)

Flowcytometry is a technique used to analyze multiple phenotypic and functional parameters simultaneously within a single cell or a population of cells. (10) Its ability to measure multiple parameters on individual cells in a suspension at high speed is ideal for the study of leukemic cells. It identifies cell markers by applying monoclonal antibodies against them. Several advances in flowcytometry, including availability of new monoclonal antibodies, improved gating strategies, and multiparameter analytic techniques, have all dramatically improved the utility of flowcytometry in the diagnosis and classification of leukemia.(11) These new applications of

flowcytometry immunophenotyping mainly rely on the concept that even if neoplastic cells show a great similarity to normal hematopoietic precursors, they frequently display aberrant phenotypes that allow their specific identification and discrimination from normal cells, even when present at very low frequencies.(12)

Materials and Methods:

This is a descriptive cross-sectional study included50 patients with acute leukemia (new cases of ALL, AML, biphenotypic acute leukemia and undifferentiating acute leukemia), who attended to the Flowcytometery laboratory in Khartoum, Sudan. The samples were fresh representative sample (more than 20% blast cells) from venous blood or bone marrow aspiration .Two ml of bone marrow aspiration was received in the hematology unit after bone marrow surgical collection in EDITAvacutte (5ml) and was mixed gently for the analysis. Laboratory analysis was performed at the Flowcytometry Laboratory using (COULTER EPICS XL-MCL™ Flow Cytometer - Miami, Florida- USA) Flowcytometer. The flowcytometery Lysing procedure for bone marrow aspiration monoclonal antibody combination was done as the follows: All tubes were labeled for analysis then pipette into each tube 20 uL of monoclonal antibody and added 100 uL of sample containing no more than 1 x 10 leukocytes / ml. The tubes were vortex for 5 seconds, then incubated at room temperature (18-25^{O}C) for 10 minutes, then added 1 ml of the "fix-and-lyse" mixture to the tube and vortex immediately for two seconds, after incubated at room temperature for at least 10 minutes, the tubes were centrifuged at 150 x g for 5 minutes and the supernatant was discarded by aspiration, then 3 mL of PBS was add, the tubes were centrifugedat 150 x g for 5 minutes and the supernatant was discard by aspiration. The pellets

were re -suspend by addition of 0.5 to 1 mL of 0.1% formaldehyde, before analysis on the flow cytometer vortex the tube for 5 seconds, then all tubes were run on flowcytometer. Statistical analysis was performed using statistical package for social science (SPSS) software (version 16 for windows 7). Evaluations of patients' data were performed using the *Chi-square* and *One way Anova*. Results with p.value< 0.05 were considered statistically significant.

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 Flowcytometer, which adjusted the cut off points between negative and positive scale for every marker, Positivity was considered when =30% of the population expressed the marker. The percentages, mean fluorescence intensity were also recorded for most of the markers.

Results:

In our study 50 patients of acute leukemia were analyzed to find out the frequency of myeloid antigen (CD13 & CD33) expression in different types of acute leukemia. Of these 29 cases were of AML, 19 of ALL, one case of Biphenotypic acute leukemia and one case of undifferentiated acute leukemia. AML and ALL cases were further subdivided into (M0 to M7) and (B-ALL and T-ALL) respectively.

Table 1: Patterns of CD13 and CD33 markers expression in Acute Leukemia

Diagnosis	No. of Cases	CD13 (%)	CD33 (%)
AML	29	19 (65.5%)	23 (79%)
ALL	19	0 (0.0%)	5 (26%)
BIPHAL	1	1 (100%)	1 (100%)
UNDIFFAL	1	1 (100%)	0 (0.0%)

Patients included 28 males and 22 females; their ages were divided into three categories, 1 - 12 years, 13 - 45 years and 46 – 77 years with minimum age of 1 year and maximum of 77 years given in (Figure.1).

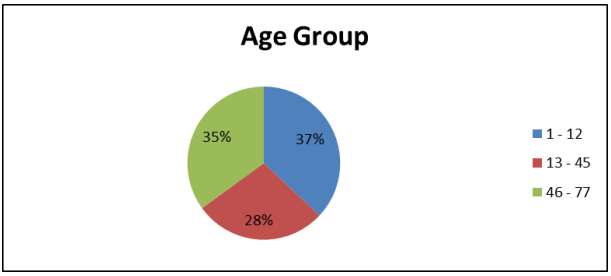

Figure (1) shows the age groups in the study population.

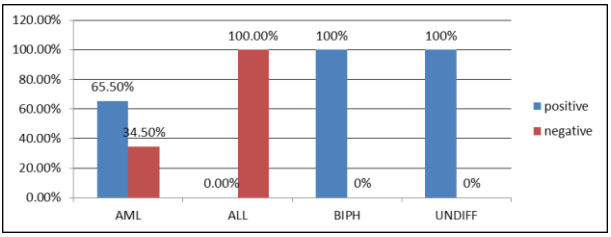

Figure (2) shows the percentage of CD13 expression in AML, ALL, BPHAL and UNDIFAL.

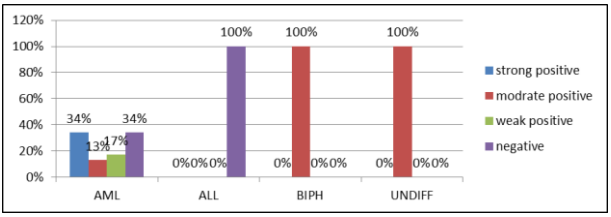

Figure (3) shows the mean fluorescence intensity of CD13 in AML, ALL, BPHAL & UNDIFAL.

Table 2: shows the CD13 &CD33 expression in AML sub groups

Sub groups of AML	No. of Cases	CD13 (%)	CD33 (%)
M0	6	4 (66%)	2 (33%)
M1	1	1 (100%)	1 (100%)
M2	7	4 (57%)	6 (85%)
M3	4	1 (25%)	3 (75%)
M4	5	5 (100%)	3 (60%)
M5	2	2 (100%)	2 (100%)
M6	1	0 (0.0%)	1 (100%)
M7	3	0 (0.0%)	3 (100%)

Table 3: shows the CD13 &CD33 expression in ALL sub groups

Sub groups of ALL	NO. of Cases	CD13 (%)	CD33 (%)
T-ALL	4	0 (0.0%)	0 (0.0%)
B-ALL	15	0 (0.0%)	5 (33%)

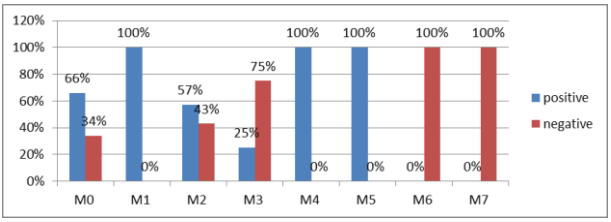

Figure (4) shows the percentage of CD13 expression in AML sub groups.

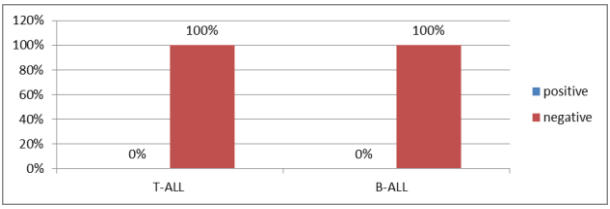

Figure (5) shows the percentage of CD13 expression in ALL sub groups.

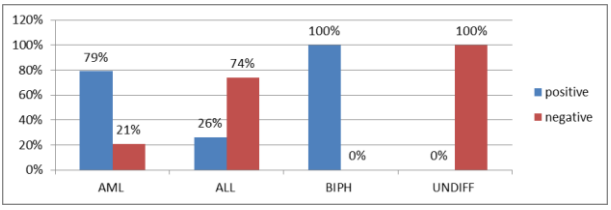

Figure (6) shows the percentage of CD33 expression in AML, ALL, BPHAL and UNDIFAL.

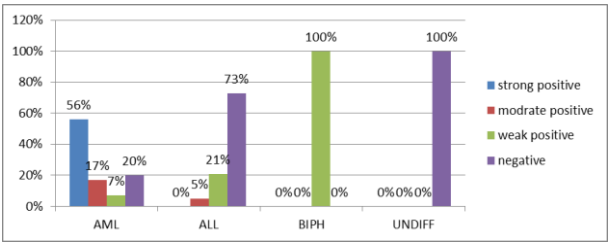

Figure (7) shows the mean fluorescence intensityCD33 in AML, ALL, BPHAL & UNDIFAL.

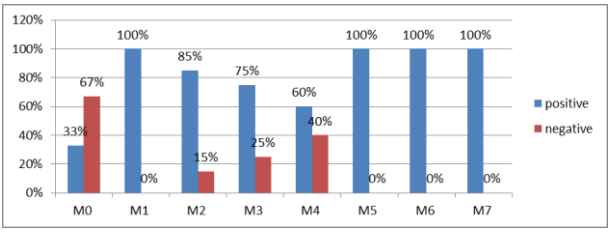

Figure (8) shows the percentage of CD33 expression in AML sub groups.

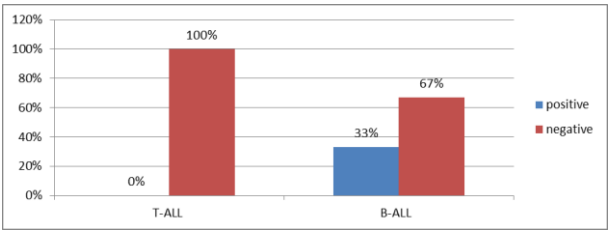

Figure (9) shows the percentage of CD33 expression in ALL sub groups.

Discussion:

Detection of CD13 and CD33 expression have a remarkable importance not only for accurate diagnosis and identification of acute leukemia but also in the sub classification of acute myeloid leukemia .In current study, we analyzed 50 cases of acute leukemia using multiparameter Flowcytometry immune phenotyping.

The expression of CD13 and CD33 among the sub groups of AML showed in (Table 2), we found that CD13 was expressed early in hemopoietic cells ontogeny in the lesser-differentiated acute myeloid leukemia subtypes, including FAB M0, M1, M2, M3, M4 and M5. CD33 was expressed in all sub groups of AML; this finding confirmed that CD33 is a pan myeloid marker can be used in the identification of AML cases. Our findings were compatible with*Nada Kraguljac*et al who had reported that CD13 and CD13 are expressed very early in differentiation process of myeloid lineage. (14)

The expression of CD13 and CD33 among the sub groups of ALL (Table 3) showed thatCD13 was negative in both T-ALL and B-ALL. In some recent studies by Boucheix and Preti, they reported that the presence of CD13 and CD33 antigens was observed on less than 10% of ALL patients. (15, 16) We found that CD33 was negative in T-ALL and positive in B-ALL (33%), so they commented that in B- ALL showed some aberrant

expression antigen expression using antibodies in only two cluster groups (CD13 and CD33).[17]

In this study flowcytometry patterns of CD13 and CD33 antigen expression showed that all of the remark results, marker percentage and mean fluorescence intensity were highly significant in the differentiations between AML and ALL cases which mean that we can use CD13 and/or CD33 in the identification and diagnosis of AML cases and sometimes in the exclusion of some ALL cases in which CD33 present as aberrant expression.

CD13 showed considerable positive expression in Biphenotypic acute leukemia and undifferentiated acute leukemia cases, while CD33 was negative with undifferentiated acute leukemia cases. Therefore, the negativity of CD13 result can be used in the exclusion of Biphenotypic acute leukemia and undifferentiated acute leukemia.

Regarding the flowcytometry parameters, we found that CD13 percentage level and mean intensity showed a significant differentiation between sub group of AML (p.value= 0.05 & 0.038, respectively) while they were insignificant in the differentiation between sub types of ALL (p.value = 0.36 & 0.48, respectively). CD33 just showed a significant differentiation between sub types of ALL (p.vale = 0.024) due to its expression with some cases of B-ALL while showed insignificant relations with the other parameters.

Conclusion:

We concluded that CD13 and CD33 are specific markers for myeloid lineage with some aberrant expression in other types of acute leukemia among the Sudanese population. This expression of CD markers can be due to their normal genetic programmed, but also the neoplastic transformation might have affected antigen expression patterns. The presence of aberrancy

helps to identify a neoplastic process and accurate diagnosis. Regardless of the CD13 and CD33 antigen expression, further studies of such cases may improve our understanding of gene expression.

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