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The properties of CD45/ SS in the blast Population of AML Sudanese patients

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Abstract:

Background: *Leukemia is a group of disorders characterized by production of excessive numbers of abnormal white blood cells in the bone marrow and blood. Acute leukemia is a rapid progressive and fatal leukemia. Acute Myeloid Leukemia (AML) accounts for*

approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. Immunophenotyping has become extremely important not only in diagnosis and sub classification of AML but also in the detection of the minimal residual disease. The aim of this paper was to study the role of CD45 gating strategy and its properties using flowcytometry in acute myeloid leukemia and to achieve good discrimination between the blast cell population and the normal cells using the flowcytometer technique.

***Method**: This is descriptive cross sectional study involved 49 patients newly diagnosed as acute myeloid leukemia (AML). The tubes were labeled for analysis. 20 µL of monoclonal antibody (CD45) was added into each tube. 100 µL of sample was added containing no more than 1 x 104 leukocytes / ml. Each tube was vortexed for 5 seconds. The tubes were incubated at room temperature in dark place for 10 minutes. 1 ml of RBCs lysis was added into each tube then incubated at room temperature in dark place for 10 minutes. All tubes were run by flowcytometer. The flowcytometric parameters (Percentage and mean fluorescence intensity) were recorded by SYSTEM II software.*

***Results and discussion:** In this study, we demonstrate the usefulness of a simple and reliable flowcytometry method to guide the classification of AML. We observed the age group (46 - 70) years has most frequent of AML cases. Male and female had equal frequency and the most samples were bone marrow. In CD45 percentage degree among the AML sub types, we found that CD45 was insignificant to differentiation between the AML types (p.value = 0.441). Regarding the CD45 mean intensity, In our study we found that all M1, M6 & M7 cases showed dim expression of CD45 with no moderate expression. While all M5 cases showed moderate. That mean we can use CD45 min intensity to differentiate between M5 and (M1, M6, M7) cases especially with negative or weak Sudan Black B stain (significant P. value = 0.002) .We observed in CD45 peak width that most of AML sub types showed heterogeneous results except some few cases with M0, M2, M4 and M5. Finally we observed the most SS peak width were heterogeneous (significant p. value = 0.05).*

***Conclusion**: In our study, we found that CD45 showed a significant role in the diagnosis and differentiation of sub types of*

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acute myeloid leukemia cases in Sudanese patients. These finding may help in the minimization of monoclonal antibodies panel especially with countries of limited recourses like Sudan.

Key words: CD45/SS, AML Sudanese patients

Introduction

Acute myeloid leukemia, characterized by circulating neoplastic cells in the bone marrow but also encompassing similar cases in which there are neoplastic cells in the peripheral blood[1]. It is a common hematological malignancy, accounts for approximately 80% of acute leukemia in adults and 20% of acute leukemia in children [2]. 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 [3].

CD45 is a protein tyrosine phosphates' (PTP) located in hematopoietic cells except erythrocytes and platelets. CD45 is also called the common leukocyte antigen [4]. CD45 is a protein that has several isoforms and the hematopoietic cells express one or more of the isoforms. The specified expression of the CD45 isoforms can be seen in the various stages of differentiation of normal hematopoietic cells [5].

The ability of flow cytometry to identify myeloid versus lymphoid differentiation approaches 98%.However, the prognostic value of immunophenotypic data is controversial [6-7]. The role of flowcytometry in sub classification of AML was improved by utilization of a variety of gating strategies including the use of CD45/side scatter gating [8].

Materials and methods

This is a descriptive cross-sectional study conducted in Khartoum state; patients attended the Flowcytometry laboratory in the period from April 2015 till Jun-2015.

This study involving 49 patients newly diagnosed as acute myeloid leukemia (AML). The tubes were labeled for analysis. 20 µL of monoclonal antibody (CD45) (Immunostep, Salamanca, Spain) was added into each tube. 100 µL of sample was added containing no more than 1 x 104 leukocytes / ml. Each tube was vortexed for 5 seconds. The tubes were incubated at room temperature in dark place for 10 minutes. 1 ml of RBCs lysis was added into each tube then incubated at room temperature in dark place for 10 minutes. All tubes were run by flowcytometer (Epics XL-MCL Beckman Coulter, Miami, USA). The flowcytometric parameters (Percentage and mean fluorescence intensity) were recorded by SYSTEM II software.

Results:

Out of the 49 cases diagnosed as AML analyzed by flowcytometer. Our findings showed that (20%) in the age group of (0.5-12) year, (44%) in (13-45) year group and (36%) in (46-70) year group. Males were (50%) of study population and females were (50%). (62%) of samples were bone marrow aspirate and (38%) were peripheral blood samples. AML sub types findings were: M0 (20%), M1 (4%), M2 (36%), M3 (12%), M4 (10%), M5 (12%), M6 (2%), M7 (4%) (Figure 1). CD45 percentage degree findings were: M0 results: negative (20%), low (20%), moderate (40%), high (20%). M1 results: negative (50%), high (50%), M2 results: negative (16.6%), low (38,8%), moderate (16.6%), high (27.7%), M3 results: low (33.3%), moderate (16.6%), high (50%), M4 results: negative (60%), low (20%), moderate (20%). M5 results: negative (33.3%), low

(16.6%), moderate (16.6%), high (33.3%). M6 results: high (100%). M7 results: low (50%), moderate (50%) (Figure 2). CD45 mean intensity findings were: M0 results: dim (70%) and moderate (20%). M1 result: dim (100%). M2 results: dim (72%) and moderate (16%). M3 results: dim (83%) and moderate (17%). M4 results: dim (20%) and moderate (80%)|. M5 results: (0%) and moderate (100%). M6 results: dim (100%). M7 result: dim (100%) (Figure 3). CD45 peak width results: M0 results: homogeneous (10%) and (90%) heterogeneous. M1 result: heterogeneous (100%). M2 results: homogeneous (11%) and heterogeneous (89%). M3 results: homogeneous (100%). M4 results: homogeneous (80%) and heterogeneous (20%). M5 results: homogeneous (66%) and heterogeneous (33%). M6 result: homogeneous (100%). M7 result: heterogeneous (100%) (Figure 4). Side Scatter (SS) mean intensity results were: M0 results: low (90%) and high (10%). M1 results: low (50%) and high (50%). M2 results: low (72%) and high (28%). M3 result: high (100%). M4 results: low (80%) and high (20%). M5 results: low (17%) and high (83%). M6 result: low (100%). M7 result: low (100%). Side Scatter (SS) peak width results were: M0 results: homogeneous (10%) and (90%) heterogeneous. M1 result: heterogeneous (100%). M2 results: homogeneous (5%) and (95%) heterogeneous. M3 result: heterogeneous (100%). M4 results: homogeneous (60%) and heterogeneous (40%). M5 results: homogeneous (17%) and heterogeneous (83%). M6 result: heterogeneous (100%).M7 result: heterogeneous (100%).

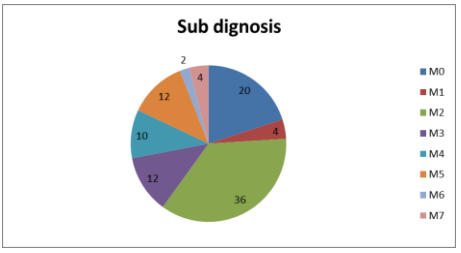

Figure 1: Shows AML sub types in the study population

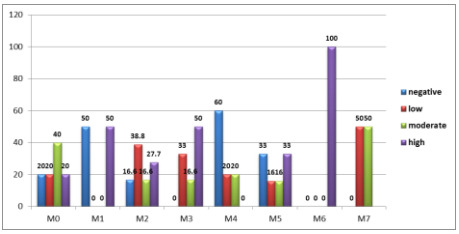

Figure 2: Shows CD45 percentage degree among AML sub types

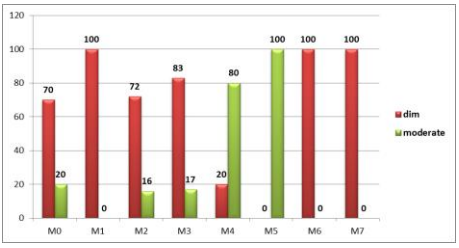

Figure 3: Shows CD45 mean intensity among AML sub types

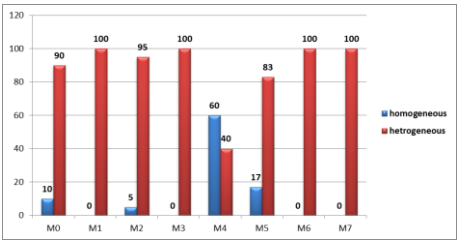

Figure 4: Shows CD45 peak width among AML sub types

Discussion

In this study, we demonstrate the usefulness of a simple and reliable flowcytometry method to guide the classification of AML. We observed the age group (46 - 70) years has most frequent of AML cases. Male and female had equal frequency and the most samples were bone marrow. In CD45 percentage degree among the AML sub types, we found that CD45 was insignificant to differentiation between the AML types (p.value = 0.441). Regarding the CD45 mean intensity, In our study we found that all M1, M6 & M7 cases showed dim expression of

CD45 with no moderate expression. While all M5 cases showed moderate. That mean we can use CD45 min intensity to differentiate between M5 and (M1, M6, M7) cases especially with negative or weak Sudan Black B stain (significant P. value = 0.002) .We observed in CD45 peak width that most of AML sub types showed heterogeneous results except some few cases with M0, M2, M4 and M5. Finally we observed the most SS peak width were heterogeneous (significant p. value = 0.05). This finding was support that CD45 / SS can help in the differentiation by different type of blast depend upon the location of population in the CD45/SS histogram. Our results were agree with F Lacombe, et al study whose reported that the CD45/SSC gating procedure improved phenotypic determination of the blast cells [9].

Conclusion:

In our study, we found that CD45 showed a significant role in the diagnosis and differentiation of sub types of acute myeloid leukemia cases in Sudanese patients. Multi-parametric analysis of CD45/Side Scatter highlighted a lot of advantages assist in the early and accurate differentiation. These benefits may help in the minimization of monoclonal antibodies panel especially with countries of limited recourses like Sudan.

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