ORIGINAL RESEARCH PAPER

Volume-8 | Issue-7 | July - 2019 | PRINT ISSN No. 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

USE OF FLOW CYTOMETRIC SSC VS CD45 PLOT TO DIFFERENTIATE BETWEEN ACUTE LEUKEMIAS

Pathology

Dr. Vijay Mulay Associate Professor, Pathology Department, Government Medical College and Hospital, Aurangabad

Dr. Anuprita Kulkarni* Junior Resident III, Pathology Department, Government Medical College and Hospital, Aurangabad *Corresponding Author

Dr. Vaidik Shrivastav Assistant Professor, Pathology Department, Government Medical College and Hospital, Aurangabad

Dr. Anil Joshi Professor and Head, Pathology Department, Government Medical College and Hospital, Aurangabad

ABSTRACT

INTRODUCTION- Flow cytometry is an important diagnostic tool in confirming and typing acute leukemias. In a setup where limited number of antibodies are available, preliminary graph of SSC Vs CD45 can be helpful in differentiating acute lymphoblastic leukemia and acute myeloid leukemia.

OBJECTIVE: To distinguish between acute leukemias using SSC vs CD45 plot.

METHOD- Clinical features, PS and BM aspirate findings and flow cytometry of all the patients were performed. The main parameter was to study the SSC vs CD45 plot. The width, height, width/height ratio (w/h) of the blast cluster was calculated. 50 cases were studied. 19 cases were ALL and 31 were AML. The w/h ratio in ALL cases was >1.6 and in cases of AML it was <1.6.[1]

CONCLUSION- The visual shape and w/h ratio of the blast cluster in SSC vs CD45 plot can be used as a preliminary parameter to distinguish between ALL and AML.

KEYWORDS

Flow cytometry has become an important diagnostic tool in the confirmation and typing of acute leukemias. The Side scatter Vs CD45 plot is a preliminary plot in the diagnosis of leukemias. CD45 also called "Leukocytes Common Antigen" is expressed on mature and immature hematopoietic cells with the exclusion of platelets, erythrocytes, elements belonging to the erythroid compartment, and highly immature hematopoietic precursors.[3] Blasts show dim positivity to CD45 and they have a low side scatter. The shape of the blast cluster when gated on SSC Vs CD45 plot can give us a preliminary diagnosis of the type of acute leukemia.

MATERIALS AND METHOD

A sample size of 50 patients who were newly diagnosed with acute leukemia in Government Medical College and Hospital, Aurangabad was selected. The clinical history included age, gender, pallor, fever, lymphadenopathy, splenomegaly, bleeding tendencies etc. The complete blood count reports were studied. A primary diagnosis of acute leukemia was made based on the peripheral smear and bone marrow aspirate examination. Further work up with flow cytometry was advised.

Peripheral blood or bone marrow aspirate samples were collected for immunophenotyping by flow cytometry. Samples were processed by lyse-stain-wash method and acquired on Beckman Coulter NaviosX Flow Cytometer.

The panel of antibodies included CD45, CD34, CD20, CD19, CD10, CD1a, CD7, CD3, CD4, CD8, CD5, CD56, CD16, CD64, CD13, CD33, CD117, cCD79a, cCD3, antiMPO. Blasts were gated on SSC Vs Cd45.

The side scatter was plotted on Y-axis and CD45 plotted on X-axis. The SSC Vs CD45 plots of all patients were copied with maximum dimensions on A4 size paper. The shape of the blast cluster was noted. Maximum width (w) and maximum height (h) of the blast cluster was recorded. The w/h ratio for each patient was noted. The cut-off for width/height ratio (w/h) was set to be 1.6.[1]

Final diagnosis was made on the basis of clinical features, complete blood count, peripheral smear and bone marrow aspiration findings, cytochemistry and immunophenotyping by flow cytometry.

RESULTS

In total 50 cases were studied. These included the age group from 1 year to 70 years. Out of the fifty cases, 29 were males and 21 females. All were newly diagnosed cases of Acute leukemia. 19 cases were found to be of Acute Lymphoblastic Leukemia and 31 cases were of Acute Myeloid Leukemia.

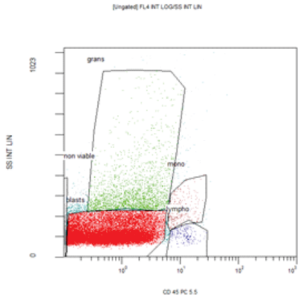

Fig. 1 Representative SSC Vs CD45 in ALL

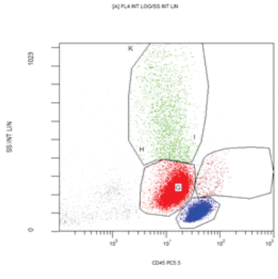

Fig. 2 Representative SSC Vs CD45 in AML

Table 1. w/h ratio of the blast cluster in cases of Acute Leukemia

	w/h ratio >1.6	w/h ratio <1.6
ALL	18	1
AML	1	30
Total	19	31

18 out of 19 cases of ALL showed width:height (w/h) ratio of >1.6, average was 2.23 . The w/h ratio of 30 out of 31 cases of AML was <1.6 with an average of 0.75.

DISCUSSION

Hematological malignancies like acute leukemias are occasional diagnosis in health care. Nowadays, confirmation of acute leukemias is mainly done by flow cytometry after a primary morphological diagnosis on peripheral smear and/or bone marrow aspirate smears. In a government set up it is difficult to match the cost of flow cytometry antibodies. In such situations it is important to find effective ways to reduce the cost of a diagnostic test.

SSC Vs CD45 plot is a preliminary plot in the diagnosis of acute leukemia. The blasts or immature cells have low side scatter and they show dim positivity to CD45. Haycocks et al[6] suggested that SSC Vs CD45 panel could distinguish between myeloblasts and lymphoblasts.[6]

In our study, 50 cases of acute leukemia were discussed. Primary diagnosis was made on the basis of clinical features, complete blood counts, morphological findings of peripheral smears and bone marrow aspirate. Flow cytometry was used to confirm the diagnosis of acute leukemias. Blasts were gated on SSC Vs CD45 plot. The maximum width and maximum height of the blast cluster was measured and the width:height ratio was calculated.

According to Saksena et al, the w/h ratio cut off was set to be 1.6.[1] The w/h ratio in 18 out of 19 cases of ALL was found to be >1.6. Whereas the w/h ratio was <1.6 in 30 out of 31 cases of AML., The result of our study was in accordance to the finding of Saksena et al.

CONCLUSION

The preliminary plot of SSC Vs CD45 helps to gate the blasts apart from the normal leukocytes. The width/height ratio of this blast cluster with a cut off of 1.6 can be used to classify acute leukemias into acute lymphoblastic and acute myeloid leukemia. The visual shape and w/h ratio of the blast cluster in SSC vs CD45 plot can be used as a preliminary parameter to distinguish between ALL and AML and further a specific antibody panel can be added to confirm and type the acute leukemias.

REFERENCES

1. Annapurna Saksena, Parul Gautam, Parth Desai, Naresh Gupta, A. P. Dubey, and Tejinder Singh, Side scatter versus CD45 flow cytometric plot can distinguish acute leukaemia subtypes, Indian J Med Res. 2016 May; 143(Suppl 1): S17–S22
2. Lacombe F, Durrieu F, Briais A, Dumain P, Belloc F, Bascans E, Reiffers J, Boisseau MR, Bernard P, Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia, Leukemia. 1997 Nov;11(11):1878-86.
3. Brahimi M, Saidi D, Touhami H, Bekadja MA (2014) The Use of CD45/SSC Dot Plots in the Classification of Acute Leukemias. J Hematol Thromb Dis 2: e107
4. Li W1, Chen Z, Liu Z, Zou P, Application of CD45/SSC gating multiparameter flow cytometry in the classification of acute leukemia--an analysis of 139 cases. J Tongji Med Univ. 2001;21(3):209-11.
5. Haycocks NG, Lawrence L, Cain JW, Zhao XF. Optimizing antibody panels for efficient and cost-effective flow cytometric diagnosis of acute leukemia. Cytometry B Clin Cytom. 2011;80:221–9.