

Immunophenotyping of Peripheral Blood and Bone Marrow Cells by Flow Cytometry

*Akanni EO and #Palini A.

* Department of Haematology & Blood Transfusion, College of Health Science, Ladoko Akintola

University of Technology, P.M.B 4400, OS 230001, Osogbo . Nigeria

* olufemiakanni@yahoo.com

Flow Cytometry Section, Central Blood Transfusion Centre,
Hospital San Raffaele, Milan, Italy

Introduction

Immunophenotyping describes a process used to identify cells, based on the types of antigens or markers on the surface of the cell. The process is used to characterize and diagnose specific types of leukemia and lymphoma by comparing the cancer cells to normal cells of the immune system (1). The method uses the reaction of antibodies with cell antigens to determine a specific type of cell in a sample of blood cells, marrow cells or lymph node cells. The antibodies react with specific antigens on the cell. A tag is attached to the antibody for its detection (2) which can be identified and quantified by a flow cytometer.

Flow cytometry is used to identify a cell population of interest by exploiting its characteristic differential light scattering and immunofluorescence. One of the principal applications of flow cytometry has been the identification and the quantitative analysis of lymphocyte subsets (3). It is therefore possible to accurately distinguish lymphocytes from other leukocyte populations in the peripheral blood using the combination of fluorescence associated with CD45/CD14 and forward and orthogonal light scatter (4).

Flow cytometry has now become the preferred method for lineage assignment, maturational characterisation of malignant cells, detection of clonality, heterogeneity and aberrant features of the malignant cell populations and quantitation of haematopoietic cells (5).

Materials and Methods

Monoclonal antibodies: CD45, CD3, CD4, CD8, CD16+56, CD19 etc conjugated to fluorescein Iso-thiocyanate (FITC), Phycoerythrin (PE), Peridine and chlorophyllprotein (Per CP), FCD, Allophycocyanin (APC), PE-CY5, PE-CY7 obtained from various sources such as Becton Dickinson, CA, Beckman Coulter, etc .

Cell preparation and Immunofluorescence: Blood and bone marrow aspirates were obtained from consenting normal adult volunteers and suitable donors serving as base line controls. Pre- and post- transplantation blood, stem cells, and bone marrow samples were also

obtained from patients in the haematology and oncology departments of the Hospital San Raffaele, Milan, Italy.

Aliquots of whole blood specimens were stained with pre-determined volumes of previously tested and titred monoclonal antibodies specific for each phenotype marker. After staining, the red cells in each tube were lysed and the remaining cells fixed with paraformaldehyde. The prepared specimens were analysed on a flow cytometer.

Flow cytometry analysis: Quantitative fluorescence analysis was performed using a multiparametric flow cytometry analyser of Beckman-Coulter, Cytomics FC 500, Florida, and USA. Samples were analysed as follows:

For each specimen, values were determined as cells/microlitre of blood expressing a particular phenotype. The "single platform" method was used to determine absolute counts. This procedure employed an internal standard of fluorescent microbeads. The number of cells having each phenotype was determined independently. In addition to determining the absolute lymphocyte counts the panel of tests contained monoclonal antibodies identifying cells bearing the following phenotype markers: CD3, CD16/56, CD8, CD4, CD45, CD14 and CD19.

During analysis in the flow cytometer, lymphocytes were identified and electronically gated on light scatter and fluorescence signals. Cells binding the relevant markers were identified by their fluorescence signals.

Below are typical flow cytometry analysis histograms:

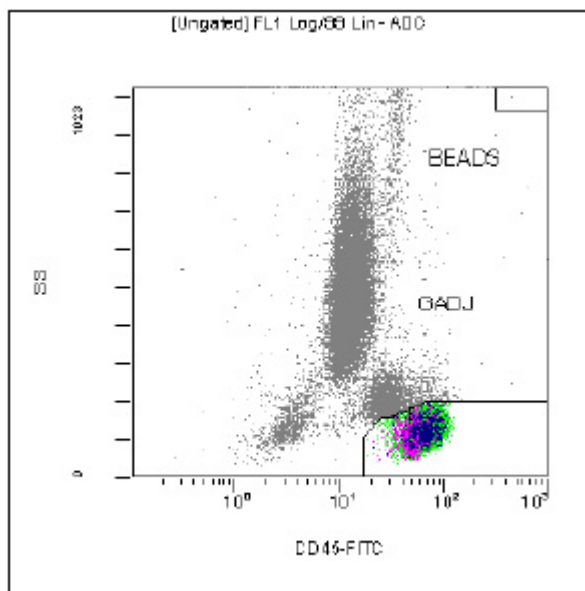


Figure 1. Bivariate display of CD45 \square FITC fluorescence versus side scatter. Lymphocytes that express bright CD45 are electronically gated. All other cells are excluded from further analysis

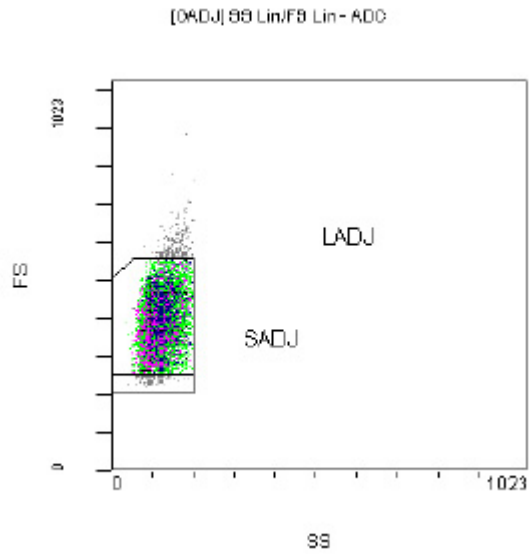


Figure 2. Bivariate display of orthogonal (side) scatter versus forward light scatter to determine the purity and the percentage (or yield) of the gated lymphocytes

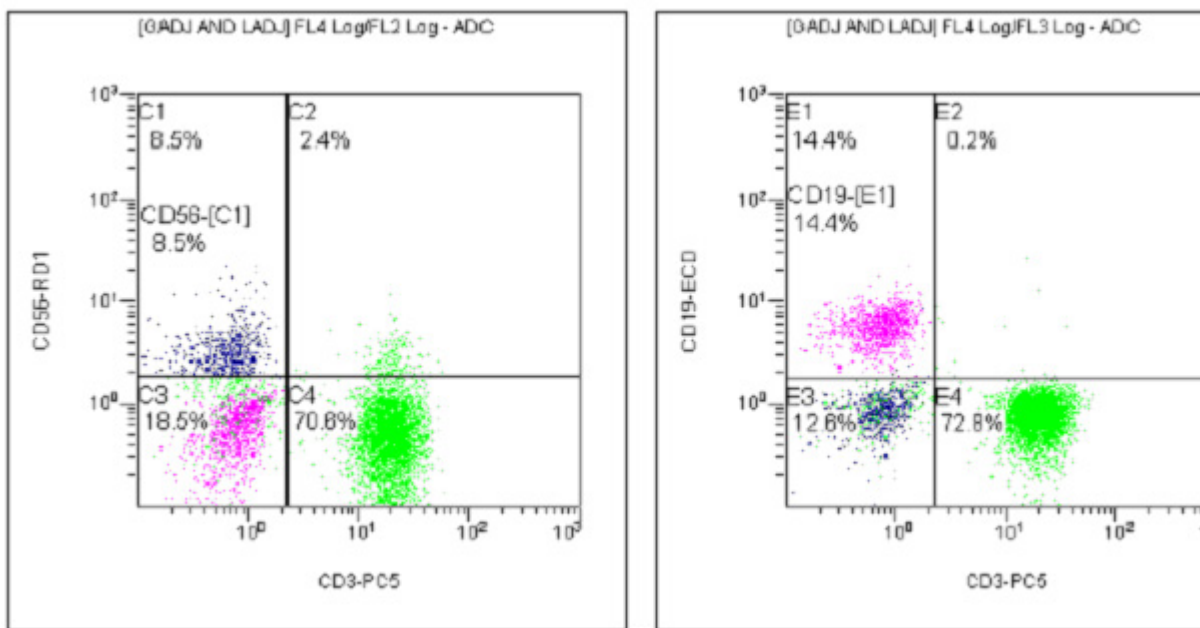


Figure 3 - 4. Bivariate fluorescence displays of CD3 versus CD56 and CD3 versus CD19. Integration cursors are placed to enumerate the percentage of cells in each sub-population of lymphocytes

tetraCXP	2.0		
IDENT	PERCENT	CELLS/ μ L	NUMBER
CD3+	72.98	1387	5348
CD19+	14.44	274	1058
CD3-CD56+	8.50	162	623
T+B+NK	95.92		

Figure 5. Statistics Box: Sum of Lymphocyte subsets (L-Sum) as an internal control. Percentage of T-Cells + B-Cells + NK Cells should add up to 90-100%

Discussion and Conclusion

The enumeration and characterization of lymphocyte subsets are crucial in specific clinical situations; hence, strategies to perform reliable counts are most important. Flow cytometry therefore provides a specific means of identification and quantitation of cell population of interest with the highest sensitivity. The reliability of the count, however, depends on the instrument performance, reagents and gating strategies.

Flow cytometry offers the best approach for evaluating multiple antigens simultaneously on large numbers of cells in a short time. In view of its requirement for a mono-dispersed cell suspension, flow cytometric identification of antigens has had its greatest impact in Haematology and Immunology. Several of these applications, such as the enumeration of CD34+ haematopoietic progenitor cells in samples that will be used for transplantation; have proved to be of great clinical utility.

Apart from the analysis of normal cells, clinically useful applications of flow cytometry to immunophenotyping have also extended to the identification and study of pathologic leukocytes and other blood cells in several different disease conditions.

Recent reports have also shown that flow cytometry immunophenotyping is well suited for rare-event analysis; the immunophenotypic identification, enumeration and characterisation of human mast cells and dendritic cells.

Acknowledgement

I would like to thank the International Federation of Clinical Chemistry (IFCC) for supporting this program and the entire staff of the Central Blood transfusion centre /Flow cytometry section of the Hospital San Raffaele Milan, Italy for their cooperations.

References

- Cancer dictionary and Acronyms. Web definitions for immunophenotyping. <http://www.dictionary.rare-cancer.org>
- Chronic Lymphocytic Leukemia Research Consortium, Glossary I. <http://www.cl.lucsd.edu/glossary1.htm>
- Loken MR: Cell surface antigen and morphological characterisation of leukocyte populations by flow cytometry. In: Methods in Haematology. Beverly P (ed) Churchill Livingstone, London 1985, 132-144.

Loken M, Brosnan J, Bach B and Ault K. Establishing optimal lymphocyte Gates for Immunophenotyping by flow cytometry. *Cytometry* 1990; 11:453-9.
European Working Group on Clinical Cell Analysis (EWGCCA). Consensus Document on Leukemia Immunophenotyping (1996).