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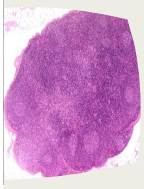


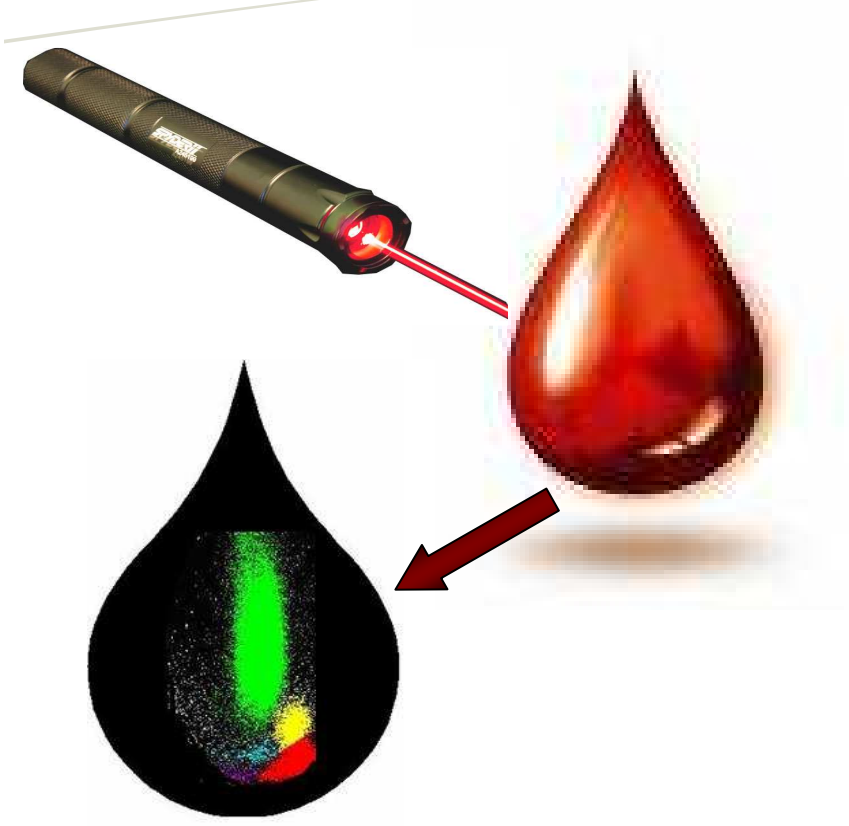
▶ FLOW CYTOMETRY ON LYMPH NODES .....4.

▶QUIZ TIME.....12

ISSUE 01 VOLUME 01 APRIL 2009

FLOW focus

Clinical Flow Cytometry e -Newsletter



INSIDE THIS ISSUE

TRYST WITH HISTORY

FLOW CYTOMETRY ON LYMPH NODES

ROLE OF FLOW CYTOMETRIC IMMUNOPHENOTYPING IN HAEMATOLOGIC NEOPLASMS

ENHANCED SENSITIVITY DETECTION ...ARE WE READY?

JOURNAL SCAN

QUIZ TIME

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FLOW FOCUS

VOL.01, ISSUE 1, APRIL 2009

CLINICAL FLOW CYTOMETRY e-NEWSLETTER

The techniques of flow cytometry are becoming more and more important for modern diagnostic haemato-pathology. A novelty confined to a few specialized institutions as it was 15-20 years ago, it has now blossomed into a mature discipline. While the methodology is well-known, the ready availability of the equipment in the recent past, and the increasingly appreciated role it plays in clinical haematology is further helping the growth of this subspecialty. The burgeoning number of scientific articles devoted to this topic attests to the interest it has aroused as a tool for both medical research and patient care. In fact, more than a thousand such papers are now published each year. Many have contributed to the growth of diagnostic flow cytometry in India.

Publication of 'First Indian Guidelines for Immunophenotyping leukemias and lymphomas' by Sumeet Gujral, Amar Dasgupta and their colleagues about a year back provided great impetus to judicious use of data generated by this relatively novel procedure . However, we still have a long way to go. While it would be impossible to deal with all the methodologies and applications of flow cytometry currently utilized or under development, our humble effort is to propagate the information as far as and as much as we can. We hope you enjoy reading this issue as much as we did compiling it.

We solicit contributions from users of flow cytometry and sincerely hope that this e-newsletter will grow with each passing day.

Anil Handoo & Tina Dadu

For Private Circulation Only

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PREFACE

Having worked as a laboratory physician for over 50 years, I have the satisfaction of having witnessed marked advancements in technology. These advances have resulted in improved understanding of pathogenesis of most clinical conditions and pharmacokinetics of drugs. There has been a consequent development of newer diagnostic approaches and targeted medications and hence improvement in management of diseases. Development of modern medicine started decades before these 50 years. Of the many notable discoveries and advances I wish to mention a few that relate to haematology. The discovery of circulation of blood, morphological description of haemopoietic cells, measurements of cell size and their numbers, use of radioisotopes for understanding of metabolic pathways, recognition of stem cells and many others. The technological advances that made a great impact on the diagnostic methodologies include development of enzymatic kinetic measurements (with no need for protein precipitation), specific biochemical procedures and user friendly robust instrumentation with increasing automation. In the field of haematology two additional developments had the greatest impact - the electron microscopy and the measurements on large number of single cell suspensions passing through an orifice for identification of cell size, components and antigens on the cell surface. The last development occurred over 50 years ago and was applied to haematology counters by Coulter brothers. This later progressed to Fluorescence Activated Cell Sorters (FACS).

The scientific potential of FACS as a research tool or its utility to assist in diagnosis is growing every day. As compared to any other technique flow cytometry is several folds faster and the number of cells that are analysed is many fold. Newer instruments can simultaneously measure 12 fluorescent colors plus 2 scatter parameters. It is interesting to know that several private laboratories/hospitals have installed the flow cytometers during the last 5 years. Many more are likely to have these during the next 5 years. The diagnostic applications are also increasing rapidly, some which include: surface and cytoplasmic phenotyping for leukemias, lymphomas, PNH cells, HLA cross-match, single cell sorting for cloning, analysis of hybridomas, apoptosis, gene expression, cytokine expression, cell biochemistry, redox regulation, activated kinases and phosphatases and redox active enzymes. Furthermore, these parameters can be studied in various cells subsets without prior sorting.

With several laboratories introducing flow cytometry for diagnostic application in this country it is high time that some inputs in form of an e-newsletter are started. This would help the users to discuss their problems with each other. The activity can be extended as and when a cytometry society gets interested. I am happy that my colleagues have taken an initiative to launch it. Its success depends on the combined interest and contributions of the users of flow cytometry. It is my hope that this e-news letter will eventually blossom into an academic journal.

Dr Swaroop K Sood
Chairman Haematology/Quality Management & Coordinator Hospital Lab Services
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TRYST WITH HISTORY

While the monoclonal antibodies are being used by the flow fraternity in leukemia/lymphoma immunophenotyping using, ever wondered who led us to them? It was as part of this quest of **César Milstein (a)** that in 1975 together with **Georges Köhler (b)** (a postdoctoral fellow in his laboratory), developed the hybridoma technique for the production of monoclonal antibodies—a discovery recognized by the award of the 1984 Nobel Prize for Physiology or Medicine. This discovery led to an enormous expansion in the exploitation of antibodies in science and medicine. **Niels Kaj Jerne (c),** a Danish (English-born) immunologist shared the prize with Georges J. F. Köhler and César Milstein for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies!!!!!!!!!!!!!


a


b


c

FLOW CYTOMETRY ON LYMPH NODES

The nomenclature of lymphoid neoplasia has evolved over time and this process will certainly continue. Until recently, characterization of lymphomas and lymphoproliferative disorders was largely descriptive and based mainly on microscopic observations. However, with the current World Health Classification, diagnosis of lymphoma is not only based on morphology, but also on immunophenotype and genetic alterations, thereby making immunophenotype an integral component for proper classification. Both, immunohistochemistry (IHC) and flow cytometry (FCM) can be used for immunophenotyping, although FCM offers many advantages in comparison to IHC. In addition to having a short turnaround time, FCM (1) can analyze a broader array of antigens than IHC (2) has the ability to quantitate both, population frequencies and level of antigen expression (3) facilitates the analysis of cells within discrete subpopulations (4) allows a clear cut correlation of multiple measurements (antigen expression, DNA content, light scatter).

Flow cytometry serves a variety of roles in the field of lymphoma including:

1) **Diagnosis of Lymphomas**: One of the mainstays for establishing the diagnosis of lymphoma is to demonstrate clonality. Clonality in B-NHL is usually done by evaluating the expression of immunoglobulin kappa (κ) and lambda (λ) light chains. IHC does not usually provide a clear cut evidence of surface light chain restriction due to large amount of background immunoglobulin, which obscures the relatively weak monoclonal immunoglobulins on the surface of B cells. Not only does FCM overcome this problem, but it also evaluates the expression of kappa (κ) and lambda (λ) light chains on immunophenotypically abnormal populations, which are especially useful in lymphomas where the lymph node is, involved partially (Fig 1).
   In contrast to the straightforward B cell clonality determination, molecular analysis of T cell receptor (TCR) gene rearrangements is required to demonstrate clonality in T-NHL. Recent availability of antibodies against the variable regions of the TCR beta chain allows determination of T cell clonality by FCM.

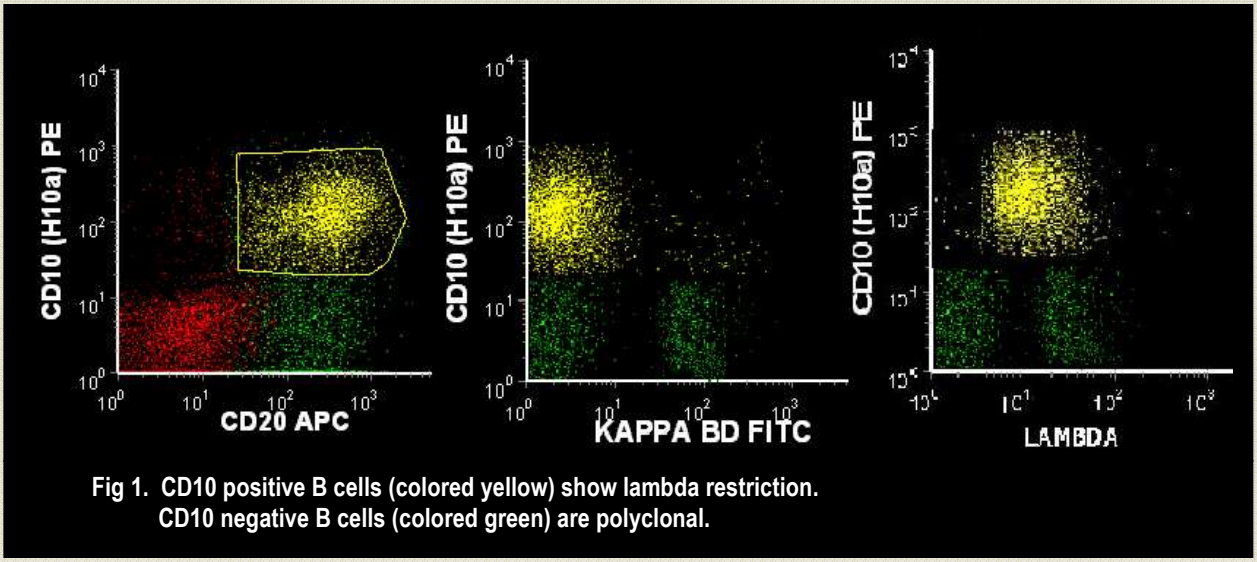
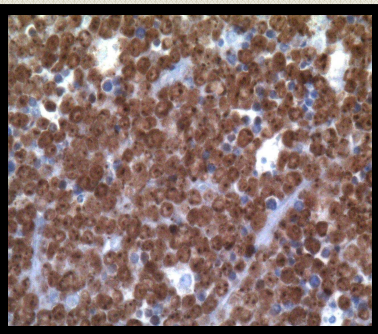


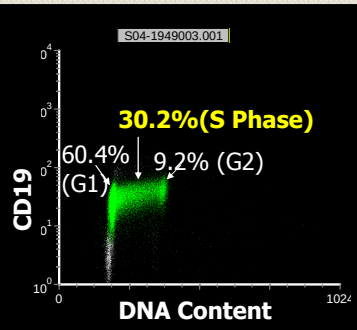
Fig 1. CD10 positive B cells (colored yellow) show lambda restriction. CD10 negative B cells (colored green) are polyclonal.

2) **Classification of Lymphomas**: The use of FCM permits simultaneous evaluation of several surface antigens, with clonality analysis providing objective and quantitative results. Most cases can be properly classified, but there undoubtedly are cases where aberrant antigen expression patterns must be reconciled with morphology.
3) **Diagnosis of Lymphoma in Small Samples**: Accurate diagnosis using FCM may be reached even on fine needle aspirates, small biopsies (mucosal by endoscopy, skin by punch), and fluids that contain small number of cells.
4) **Detection of Low Level Neoplastic Involvement**: FCM is a sensitive method for revealing small populations of abnormal lymphoid cells that may not be otherwise recognized using conventional diagnostic techniques.

5) **S-phase Fraction Analysis**: The proliferative fraction, particularly when it is measured selectively in neoplastic cells, as can be assessed by flow cytometric S-phase fraction, is a valuable complementary parameter for grading NHL.



High MIB1 in a case of Burkitt's lymphoma (IHC cannot differentiate between cells in G1, S and G2 phase)



High S-Phase Fraction (30.2%) in a case of Burkitt's lymphoma

6) **Heavy Chain Analysis**: In some atypical lymphoproliferative disorders, it is the evaluation of heavy chains by FCM that can help clinch a diagnosis

The advantages of flow cytometry are based largely on its ability to analyze, rapidly and simultaneously, multiple cell properties in a quantitative manner. As is true with any other laboratory technique, while there are many advantages, there are limitations as well, and flow cytometry is no exception. A few of the drawbacks include requirement of fresh, unfixed tissue for analysis. Also, it has had little success in the evaluation of Hodgkin lymphoma and T-cell rich B-cell lymphoma, for which morphology and IHC are superior.

Flow cytometry currently enjoys widespread use and a well established role in lymphoma diagnosis in most clinical laboratories throughout the United States and many European countries, but in India it still has a long way to go. The multiple roles that FCM plays in lymphoma diagnosis, classification and prognostication serve the current management plans and treatment protocols. The unique attributes of flow cytometry, therefore, give it a leading edge in lymphoma management.

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Raul Braylan

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Dr R. Braylan has co-authored the following book: 2007: Flow Cytometry in Hematopathology: A Visual Approach to Data Analysis and Interpretation. Second Edition. Humana Press, Inc., Totowa, N.J.

ROLE OF FLOW CYTOMETRIC IMMUNOPHENOTYPING IN HEMATOLOGIC NEOPLASMS, WITH SPECIAL REFERENCE TO MATURE B-CELL NEOPLASMS

Over the last two decades, better understanding of different clinic-pathologic and biologically distinct entities has redefined the classification of hematologic neoplasms, as exemplified in the 2001 and the current 2008 WHO schemes. Flow cytometric immunophenotyping is a critical component in the initial diagnosis and classification of these neoplasms, along with clinical findings including a complete blood count, morphologic assessment, karyotypic and relevant FISH and molecular data. This multi-parameter approach is the current standard to practice for an accurate categorization of hematologic neoplasms and guides significant treatment decisions, prognostication, establishing criteria for randomized control trials, including those offering novel therapeutic approaches, and for monitoring response to therapy.

A 2006 Bethesda conference outlined the following pragmatic consensus indications for flow cytometric immunophenotyping: cytopenias, especially bi- and pancytopenia, leukocytosis, including lymphocytosis, monocytosis and eosinophilia, presence of blasts or atypical cells in peripheral blood, bone marrow or body fluids, plasmacytosis or monoclonal gammopathy, and organomegaly and tissue masses. Publications from this consensus meeting outline reagent and antibody panels that can be designed to strategically screen for hematologic malignancies and to expand the panels for accurate classification in case of a positive screen.

Flow cytometric immunophenotyping evaluates antigenic expression (phenotype) by individual cells in a suspension. The identification of a specific phenotype is essential to lineage assignment (myeloid versus lymphoid; B versus T, etc), and for distinguishing mature from immature (blastic) populations. A detailed documentation of the pattern, intensity and uniformity of antigenic expression is helpful in identifying specific entities, narrowing down the differential diagnoses to suggest pertinent confirmatory tests, and for establishing a baseline phenotype in a patient that may be distinctive enough to allow for monitoring persistent/relapsed small abnormal populations in the future.

Flow cytometric immunophenotyping is currently most useful in the diagnosis of lymphoproliferative disorders, acute leukemias, and paroxysmal nocturnal hemoglobinuria. Role in myelodysplastic syndromes and in myelodysplastic/myeloproliferative neoplasms are less well defined. However, emerging data on these processes and increasing sophistication of flow cytometric software to assess antigenic expression patterns distinct from normal/reactive populations, hold immense promise for the future.

In the context of mature B-lymphocytic neoplasms, abnormal populations can be detected by 1) demonstrating surface immunoglobulin (sIg) light chain restriction and/or 2) by detecting aberrant antigenic expression by the B-cells. Most small lymphoid populations fall in the normal lymphocyte gate on the CD45/side light scatter co-plot. Large cell lymphomas show a high forward light scatter. Monotypic light chain expression is almost always synonymous with monoclonality and neoplasia, especially when demonstrable in the large well defined populations (caveat: rare instances of monotypic populations in clearly non-neoplastic entities like florid reactive lymphoid hyperplasia, multicentric Castleman's disease, etc, are well-documented). In situations where the monotypic population is obscured by concurrent numerically greater reactive B-cells, selective assessment of light chain expression in aberrant populations (e.g. in CD19 and CD5 co-expressing cohort of cells or, large lymphocytes on forward light scatter) can help demonstrate clear-cut light chain restriction. Some cases of mature B-cell neoplasms may not express any surface immunoglobulins (e.g, a small subset of follicular lymphoma). Evaluation with a different clone of monoclonal anti-sIg, polyclonal anti-sIg reagents, or anti-cytoplasmic light chain can be very helpful in this situation to demonstrate light chain restriction. True lack of demonstrable surface light chain expression in and of itself is an aberrant finding and highly suggestive of a clonal B-cell population

The following table outlines antigen expression patterns in common mature B-cell neoplasms.

Entities	*Characteristic immunophenotype*	*Additional diagnostic data*	*Comments*
CD5 (+), CD10 (-)			
Chronic Lymphocytic Leukemia (CLL)	Dim CD20 and sIg; CD23 (+), CD43 (+), FMC7 (-)	Characteristic lymph nodal morphology with proliferation centers	
Mantle Cell Lymphoma (MCL)	Bright CD20 and sIg; CD23(-/+),CD43 (+), FMC7 (+/-)	Cyclin D1 by immunohistochemistry or t(11;14) by FISH	
B-pro-lymphocytic leukemia (PLL)	Overlaps with CLL and MCL; bright CD20 and sIg; CD5, CD23 and FMC-7 (all +/-)	Characteristic morphology with single prominent nucleolus, high WBC count;	Vanishingly rare entity; imperative to exclude blastoid MCL, and other mimics
Notes: 1. A very small subset of diffuse large B-cell lymphoma (DLBCL) may express CD5. These may represent cases of Richter's transformation (if previous/concurrent CLL with same light chain restriction is documented) or *de novo* CD5-positive DLBCL. 2. Approximately 5% of marginal zone lymphoma (MZL) and lympho-plasmacytic lymphoma (LPL) may also express CD5. However, these processes are often readily identifiable by bright sIg and bright CD20 expression, usual lack of reactivity for CD23, and frequent plasmacytic differentiation.			
CD5(-),CD10 (+)			
Follicular lymphoma (FL)	Small/intermediate cells, usually CD43 (-)	Characteristic histology with follicular growth, bcl-2 (+), especially in grades 1 and 2.	
DLBCL	CD43 (+/-), larger cells on forward light scatter	Diffuse, large cells, bcl-2 (+/-)	
Burkitt Lymphoma (BL)	Bright CD10, CD43(+)	intermediate sized cells, bcl-2 negative, near-100% Ki-67, *c-myc* rearrangement	sIg may not be demonstrable in a subset; in these cases TdT/CD34 stain is necessary to exclude lymphoblastic leukemia/lymphoma
Notes: 1. In these CD5 (-), CD10 (+) populations, flow cytometry evaluation can be diagnostic of a clonal B-cell population of follicular center cell derivation. Further diagnosis and classification almost always would require correlation with histology to assess pattern (diffuse versus follicular) and cell size (small/intermediate versus large) and, as relevant work-up for BL if that is in the differential by morphology and immunohistochemistry. 2. A very small subset of hairy cell leukemia (HCL) and MCL can express CD10.			

CD5 (-), CD10(-)			
Marginal Zone Lymphoma (MZL)	CD11c(+/-), CD103 (-/+)	Circulating lymphocytes have larger cytoplasm, which may possess villous projections, granules; subset may have plasmacytoid appearance	Most peripheral blood cases represent circulating splenic marginal zone lymphoma; serum protein electrophoresis often positive for paraproteinemia
Hairy Cell Leukemia	Bright CD11c, bright CD22, CD25(+), CD103(+)	Characteristic morphology with reniform, monocyte-like nucleus, circumferential villous projections.	Monocytopenia common; confirm characteristic morphology in bone marrow (BM). Annexin-A1 (+)

Notes:

1. Lymphoplasmacytic lymphoma has a similar CD5 and CD10 negative immunophenotype and is closely related to MZL. Diagnosis of LPL is helped by BM biopsy, clinical correlation and documentation of IgM paraprotienemia.
2. Plasma cells (PCs) are identifiable by bright CD138, negative/very dim CD45 expression. PCs almost always are negative for CD20. CD56 expression indicates that PCs are neoplastic in origin. CD19 tends to be lost in neoplastic PCs as opposed to reactive PCs. Cytoplasmic light chain restriction is evident in clonal PCs.
3. A small subset of MCL can lack CD5. Diagnosis would depend on morphologic evaluation and demonstration of cyclin D1/t (11; 14).
4. This CD5 and CD10 negative phenotype would also include many cases of DLBCL, which again would require morphologic assessment for cell size.

Suggested Reading:

Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008; 111:3941-67

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Flowcytometric Analysis with Enhanced Sensitivity Detection - Are we ready?

The characterization of cell surface of and intracellular proteins and antigens is done effectively by immunofluorescent technique using flow Cytometry. Technologic advancement has rendered flow cytometers capable of analyzing more than 5 parameters on hundreds and thousands of cells in a few minutes. Flow cytometry, thus, has become an integral part of any hematological/immunologic diagnostic laboratory.

While the advantages of this technology are many, it has its limitations. e.g.: the type/kind of cells that can be analyzed, need for fresh specimen, availability of antibodies & sensitivity of the analytic method. Though all these and many other issues are of importance in improving and broadening the application of flow cytometry in clinical practice, the current article is an overview on techniques for enhancement of sensitivity of detection of cellular antigens using a flow cytometers. For detailed explanation please refer to the suggested readings.

The need?

By far, evaluation of hematological malignancies has been the most well documented use of flow cytometry. Clonality analysis of B- cells lymphoma has long been based on isotype exclusion of immunoglobulin (Ig) light chain expression. While there are many technical issues involving identification of light chain expression, the inherent low level expression of surface Ig on some neoplastic B-cells cannot be under-rated. For example surface Ig expression in chronic lymphocytic leukemia (CLL) is normally weak or not detected. In addition, other antigens may be expressed weakly in CLPD's including CD22, CD79b, CD5 and CD23. The latter two antigens are diagnostically critical. CD5 expression is important for distinction of CLL/mantle cell lymphoma versus non-CLL lymphoproliferative disorders and CD23 is the most reliable marker for distinction of CLL from mantle cell lymphoma.

The sensitive detection of antigens in leukemic blast-analysis (in acute leukemia), on the other hand has not taken a critical importance since low level expression of various antigens is of usual occurrence. Nevertheless, there are many reports in literature where expression of a marker was lacking on the cells but on genetic analysis identification of RNA transcripts for the same antigen could be made. Probably in such cases low level expression of the marker by flow cytometry was missed for the want of enhanced sensitivity in the detection technique. Haematopoietic stem cells are defined by the presence of CD34 antigen. Its expression is of paramount importance not only for its enumeration in stem cell transplants, but also in detecting its expression for blast identification in acute myelogenous and lymphoblastic leukemias. There is definite difference in the sensitivity of staining of CD34 as noted amongst the monoclonal antibodies because of distinct epitope reactivity. Many studies have also shown that some stem cells express low to undetectable level of CD34 marker. This low level expression can cause significant problem in stem cell enumeration, separation and purification of CD34 progenitor cells. Use of highly sensitive immunostaining technique may, thus, be required to determine the correct CD34 reactivity in the stem cells.

Studies analyzing multidrug resistance (MDR) gene products on leukemia/lymphoma cells have found it to be of prognostic importance. The detection of these gens/genes products has been hampered by many-a-factor including low level expression of multi-drug resistance proteins. A sensitive detection technique, thus, would increase the understanding of MDR expression and its function.
Example like these can be multiplied and extended to include CD5 expression of B cells subsets, CD20 expression on peripheral blood B – cells, CD20 expression on T-cells, below thresh-hold cytokine and chemokine expression, etc.

Technique(s)

The sensitivity of detecting low level antigens by flow cytometry has been targeted by many people. Many methodologies has been evolved including increasing the flurochrome to antibody ratio, use of indirect immunofluorescent staining where in additional secondary, tertiary or quaternary labeled component amplifies fluorescent signal.

Increasing the flurochrome to antibody ratio has also been improved by using liposomes as florescent-carries. These can be loaded with flurochrome in bag-like fashion and conjugated to antibodies generating an impressively sensitive detection system. Unfortunately, this approached has limited utility because of the difficulty in working with liposomes.
Recently, Kaplan et al have developed a procedure involving enzymatic amplification of fluorescent signal. This is achieved by antibody mediated localization of horseradish peroxidase into the targeted molecule. Many other techniques are currently underway and hold a lot of promise.

Are we ready?

Enhanced sensitivity of detection in flow cytometry will definitely improve our analytical capability. Beyond the enhanced ability to perform clonality assessment, the enhanced sensitivity may re-define the classic leukemia/lymphoma phenotypes; give more accurate assessment of CD34+ hematopoietic stem cells and MDR leukoblasts. But the question that haunts one's mind is: are we ready yet?
The first consensus guidelines are approximately two years back but how many of us have actually put them into practice? We are still coming to terms with standardization of techniques, finalizing minimum antibody panels, reporting issues, etc, Although the implementation of enhance sensitivity techniques in clinical flow cytometry in India is still probably a few years away, an effort to achieve it in practice can serve to identify important correlates hitherto unqualified.

Suggested Readings:

- Kaplan D, Husel W, Meyerson H. Immunophenotypic analysis with enhanced sensitivity of detection by enzymatic amplification staining. Clin Lab Med. 2001 Dec;21(4):763-78.
- Kusenda J. Quantitative identification of blood cell markers in human hematopoietic malignancies with diagnostic and prognostic significance. Neoplasma. 2008;55(5):381-6.

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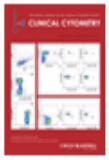
JOURNAL SCAN

Fiona E. Craig and Kenneth A. Foon. Flow cytometric immunophenotyping for hematologic neoplasms. Blood, 2008; 111: 3941 – 3967.

Gujral S, Subramanian PG, Patkar N , Badrinath Y, Kumar A, Tembhare P, Vazifdar A, Khodaiji S, Madkaikar M, Ghosh K, Yargop M, Dasgupta A. Report of proceedings of the national meeting on "Guidelines for Immunophenotyping of Hematolymphoid Neoplasms by Flow Cytometry" IJPM 2008; 51 (2): 161-166.



Gujral S, Subramanian P.G., Dasgupta A.. National Meeting on Guidelines for Immunophenotyping of Hematolymphoid Neoplasms in India. Cytometry Part B: Clinical Cytometry 2009;76B (2) : 156-157.

Al-Mawali A., Gillis D., Hissaria P., Mundy J., Lewis I. The presence of leukaemia-associated phenotypes is an independent predictor of induction failure in acute myeloid leukaemia. Int. Jnl. Lab. Hem. 2009 31(1):61-68

Fabian A, Barok M, Vereb G, Szollosi J,Die Hard: Are Cancer Stem Cells the Bruce Willises of Tumor Biology? Cytometry Part A 2009;75A: 67-74.

Lizard G. Diagnosing HIV infection using flow cytometry: From antigenic analyses to a specifically dedicated bead-based assay to measure viral load. Cytometry Part A2009; 75A: 172-174.

QUIZ TIME

76 year old male with axillary adenopathy

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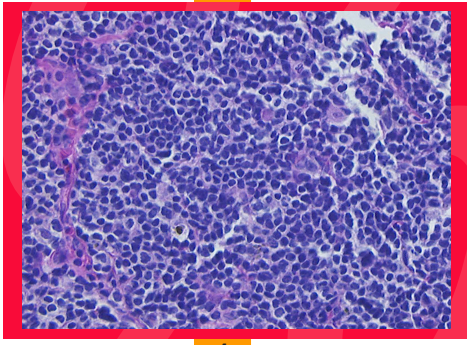

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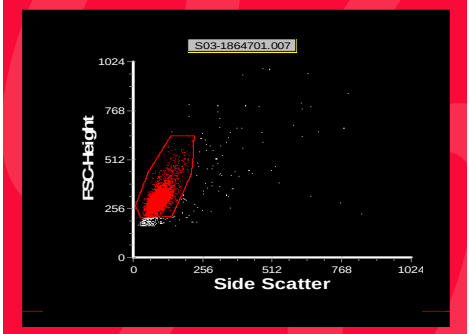

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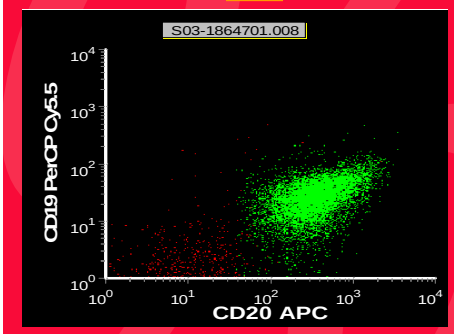

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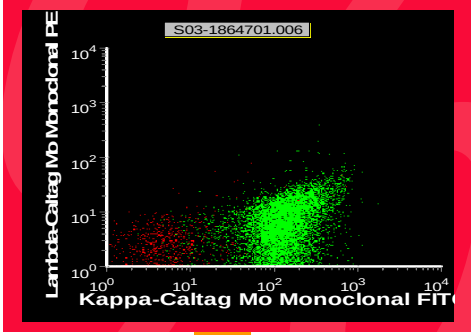

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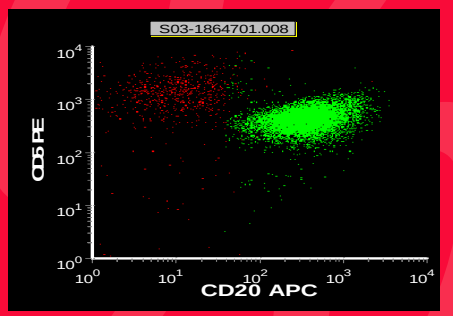

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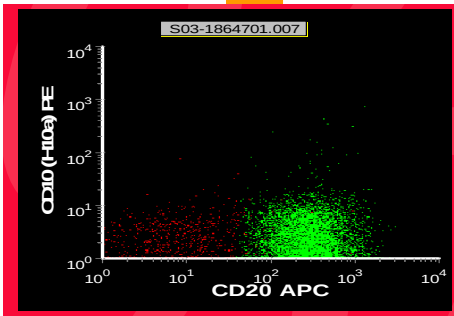

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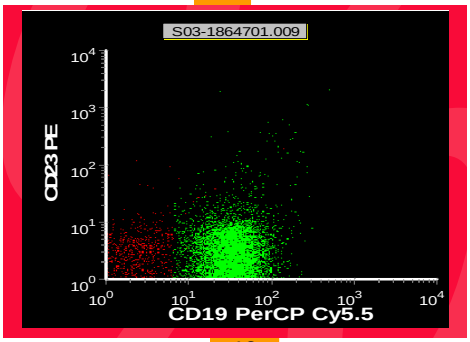

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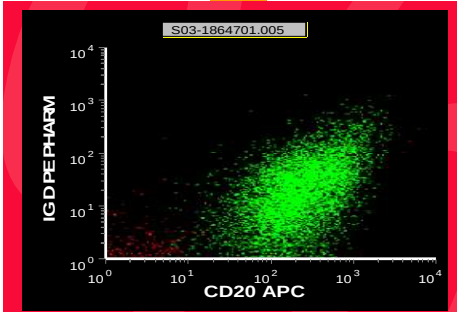

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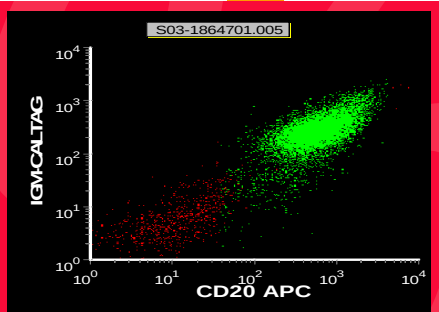

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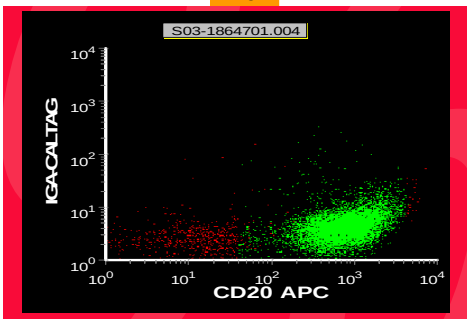

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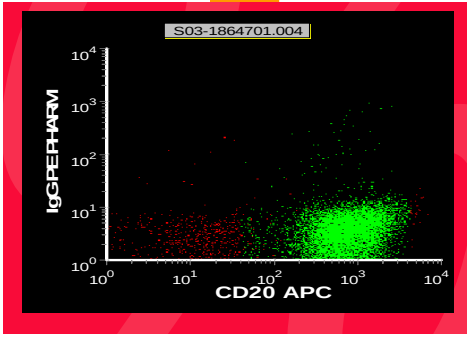

FOR ANSWER, LOG ON TO THE FOLLOWING LINK:

http://www.geocities.com/flowfocus2009/ff.html

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