Flow Cytometry in Pediatric Malignancies

ANIL HANDOO AND TINA DADU

From Centre for Exellence – Flow Cytometry – Department of Hepatology, Dr BL Kapur Memorial Hospital, New Delhi, India. Correspondence to: Dr Anil Handoo, Senior Consultant Hematology & Director, Laboratory Services, BLK Super Speciality Hospital, 5 Pusa Road, New Delhi 110 005, India. dranilhandoo@yahoo.co.in

The utility of flow cytometry as a useful diagnostic modality for the assessment of hematopoietic neoplasms has been established beyond doubt. In fact, it is now an integral part of the diagnosis and classification of various diseases like leukemias and lymphomas along with molecular studies and cytogenetics. Prognostication and disease monitoring by flow cytometry is also being recognized increasingly as one of the important fortes. This is evident by the number of articles in the published in literature on the minimal residual disease detection by flow cytometry especially in the last decade or so. To add to this, ever growing list of utilities in hematopoietic malignancies, many non-hematopoietic neoplasms can also be analyzed by flow cytometry. The examples include fluid specimens from serous cavity effusions and samples from solid tissues like lymph nodes, reticulo-endothelial tissue, central nervous system tissue, etc. Flow cytometry technique provides a unique blend of rapidity, high sensitivity and specificity compared to cyto-morphology and conventional immunohistochemical staining. It is also remarkable for simultaneous analysis of more than one marker on the cells. Evaluation of limited samples such as cerebrospinal fluid or fine needle aspiration samples makes Flow cytometry a valuable tool. DNA ploidy analysis and assessment of pediatric non-hematopoietic neoplasms by Flow cytometry has envisaged the utility vista of this technique. This review is aimed at providing an insight into the applications of flow cytometry in pediatric malignancies.

Keywords: *Diagnosis, Immunophenotyping, Lymphoma, Leukemia.*

Pediatric malignancies, relatively rare and heterogeneous group of hematological and non-hematological malignancies, require a multi-modality approach for its diagnostic screening and classification [1,2], which in turn helps in appropriate therapy and better outcomes. The need for single cell suspensions in flow cytometry technique has limited its use for fluid samples, primarily blood and bone marrow. Therefore, multiparameter flow cytometric immuno-phenotyping has been primarily used to provide rapid diagnosis and help classify most of the hematological malignancies, including pediatric leukemias and lymphomas, where there is blood and/or marrow involvement, as well as body fluid samples like cerebrospinal fluid, pleural fluid and peritoneal fluid, etc. Pediatric solid tumors, like small round cell tumors (SRCT), and nodal lymphomas are increasingly being subjected to flow cytometry, both for diagnosis and classification [3]. In addition, flow cytometry is also being used for evaluation of tumor cell DNA contents and cell cycle analysis [2].

This review is aimed at providing an insight into utility of flow cytometry in pediatric malignancies for diagnostic screening, classification, grading, prognostication, therapeutic target identification and monitoring to name a few. For simplification and better understanding we shall approach flow cytometry in hematological disorders followed by flow cytometry in solid malignancies, with focus on lymph node flow cytometry. Based on the consistency of the available sample, irrespective of whether the lesion is hematopoietic or non-hematopoietic, flow cytometry for pediatric malignancies can be classified into two types: (*i*) Flow cytometry on liquid/ fluid samples and (*ii*) Flow cytometry on tissue samples.

FLOW CYTOMETRY IN HEMATOLOGICAL MALIGNANCIES

Hematological malignancies in the pediatric age group are akin to their adult counter parts, albeit with a difference in that the lymphoid malignancies are more commonly seen and acute lymphoblastic leukemia happens to be the most common sub-group [4], with diffuse large B cell lymphoma, Burkitt's lymphoma and primary mediastinal large B-cell lymphoma being the other lymphoid malignancies encountered. Mature lymphomas, especially the low grade lymphomas are extremely uncommon and their mention is seen only as rare case reports in the literature. Flow cytometric immunophenotyping forms the cornerstone for accurate diagnosis and classification of the lymphoid neoplasms. It also forms an essential part of the armamentarium for the subsequent monitoring by way of minimal residual disease (MRD) detection. This shall be detailed in the subsequent sections through this review.

Normal Bone Marrow Differentiation Patterns

It needs to be understood that for identifying abnormal, a thorough understanding of the normal is essential. Knowledge of various cluster of differentiation (CD) markers, (common ones used in routine practice are mentioned in ***Table* I**, immunophenotypic patterns of normal hematopoiesis, especially in bone marrow samples, and reference patterns for age-related changes is pivotal for identification of abnormal [5]. Differentiation stages based on well-defined panels of monoclonal antibodies against specific antigens helps in delineating various stages of maturation. Pathological conditions like myelodysplasia or leukemia, show abnormal cells involved in abnormal differentiation pathways or arrested at a given stage of differentiation as in acute leukemias. Paediatric bone marrow samples are relatively unique in that the normal B- cell precursors are in plenty and mimic lymphoblasts both morphologically and immuno-phenotypically.

Hematogones – Mimic B Lymphoblasts

First described in the 1930s as lymphoid appearing cells in sternal marrow aspirates, hematogones, by definition, B-lymphocyte progenitor cells and mature B lymphocytes are normal bone marrow constituents [6]. They are most prominently seen in pediatric bone marrows, especially infants and in a variety of diseases in both children and adults. They are particularly elevated in regenerative bone marrows, following chemotherapy and bone marrow transplants [6]. In some instances they may be as high as more than 50% of nucleated cells. Obviously, because of their morphologic similarity with the blast cells they closely mimic acute lymphoblastic leukemias. Interestingly, even immunophenotypically, they closely resemble B- lymphoblasts, however, the maturation pattern is intact and there is no arrest at a particular stage. The entire spectrum of antigen expression that defines the normal evolution of B-lineage precursors is seen on the hematogones as depicted in the ***Table* II**; and also depicted pictorially in ***Web Fig.* 1**. The most common "J-pattern" or the "water-fall pattern" is shown.

Use of CD45 (Leukocyte Common Antigen)

Immunophenotypically leukemias can be classified acute or chronic based on relative positioning of the cells with respect to mature lymphocyte population on the CD45 side scatter (SSC) dot plot. A further insight into the data can help further subdivision into classical AML pattern, classical ALL pattern, CD45 negative pattern (ALL/ AML-M7/AML-M6/Plasma cell pattern), monocytic differentiation pattern and hematogone pattern provisional groups based on the blast distribution patterns in the CD45/SSC panel and morphology [7]. ***Fig.* 1** and ***Web Fig.* 2** shows CD45-SS dot plots with various patterns. They reveal the blasts of classical AML are usually seen as a round/oval cluster in the so called blast hole, while the blasts of a classical B cell ALL distribute horizontally along the CD45 axis with low side scatter. T cell ALL may have only a minimal to mild down regulation of CD45 (T-cell ALL pattern), and at times the side scatter is also not as low as a B cell ALL. CD45 negative pattern is usually a feature of childhood ALL. It may also be seen in cases of AML-M7/increased plasma cells and sometimes when there is large amounts of debris, which happens usually in lyse no-wash technique. A mixed CD45 negative-dim CD45 pattern is usually indicative of an erythroleukemia or mixed lineage leukemia. A typical APML pattern shows a tear drop

TABLE I COMMON CD MARKERS USED IN ROUTINE PRACTICE

Blastic	*B-Lymphoid*	*T-Lymphoid*	*Myeloid*	*Monocytic*	*Erythroid*	*Megakaryocytic*
nTdt	CD19	CD1a	cMPO	CD36	CD36	CD36
CD34	CD20	CD2	CD117	CD64	CD71	CD41
CD38	CD10	CD3	CD13	CD14	CD235a	CD42
HLA-	lambda	CD4	CD33	CD11b		CD61
DR	kappa	CD5	CD15			
	CD22 (c&s)	CD7	CD16			
	cCD79a	CD8				
		TCR-αβ				
		TCR-γδ				

n = Nuclear; c= cytoplasmic; S=Surface; CD45 (Leukocyte Common Antigen): Is the backbone for identification of various cell populations and is used as the backbone marker for leukemia/lymphoma analysis.

TABLE II IMMUNOLOGICAL MARKERS USED TO EVALUATE HEMATOGONES

Hematogones			*Mature B Cells*
Stage 1	*Stage 2*	*Stage 3*	
Tdt	-	-	-
CD34	-	-	-
CD10 (bright)	CD10	CD10	CD10
CD19	CD19	CD19	CD19
CD22 (dim)	CD22 (dim)	CD22 (dim)	CD22
CD38 (bright)	CD38 (bright)	CD38 (bright)	CD38(bright-negative)
	CD20 (dim)	CD20	CD20
	SIg	SIg	SIg

pattern of abnormal cells, starting slightly above the blast hole and going vertically upwards. But, in majority of the cases it does not touch the roof. A similar pattern is also seen in cases of chronic myelogenous leukemia with increased basophils and increased blast cells. The difference, however, is that the scatter usually starts in the blast hole and goes vertically up, extending up to the roof of the dot plot. Hematogone pattern showed cells abutting the lymphocytes with feathering into the blast region; however, they have a very low side scatter. Amongst the chronic lymphoproliferative disorders, hairy cell leukemia (HCL) has a characteristic pattern on the CD45-SS dot plot in that the neoplastic cells usually are seen on the upper pole of the normal lymphocyte cluster extending slightly upwards into the monocyte region (HCL pattern). Most of the other CLPDs are indistinguishable from the normal lymphocyte

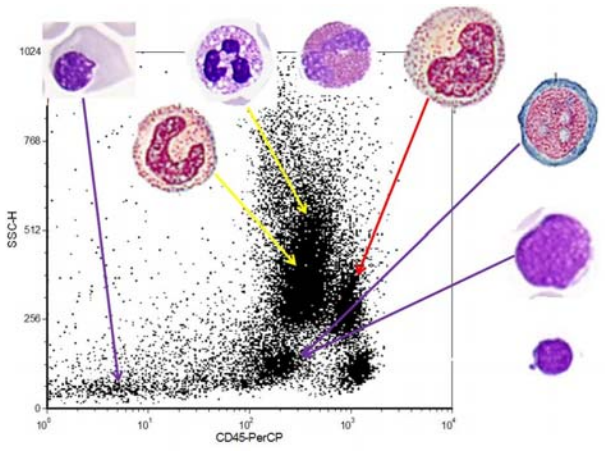


FIG. 1 *CD45-Side Scatter patterns with relative placement of various cells.*

population; however, a closer look will make one identify a minimal down regulation of CD45, so as to be able to identify a 'crescent' of normal lymphocytes (CLPD pattern) [7].

Acute Leukemia Diagnosis

While morphology continues to be the cornerstone for acute leukemia diagnosis, and presence of >20% blasts in the blood and bone marrow is suggestive of the disease, immunophenotypic features of blasts cells are pivotal in defining the stage of maturation arrest of the blast population not only within the B- and T-lymphoid lineages but for the neutrophilic, monocytic, megakaryocytic or erythroid lineages as well. As mentioned earlier, CD45 continues to be the anchoring marker to identify the presence of blast cells. The blast cells are usually dim CD45 compared to normal lymphocytes. Peculiarly, pediatric B-cell acute lymphoblastic leukemias may be dim to completely negative for CD45. Therefore, it is essential to ascertain the expression of other markers to identify the abnormal blasts. A combination of CD19 (Pan B cell marker) along with blastic markers, CD34, Tdt and HLA DR, help in delineating the blasts, in majority of the patients. CD10, CD20, CD15 are other markers which are useful in identifying B lymphoblasts [8]. An important sub-type, which is CD10 negative and CD15 positive has been seen to be associated with mixed lineage leukemia (MLL) gene abnormalities.

T cell acute lymphoblastic leukemias (T cell ALLs) on the other hand have relatively brighter CD45, sometimes as bright as normal T cells. Classically, T cell ALLs are surface CD3 negative and cytoplasmic CD3 positive; In addition most of them are usually CD4 and CD8 double negative or double positive. They can also be restricted to either CD4 or CD8, when they are classified as medullary T cell ALLs. A special mention about early precursor T cell ALL (ETP ALL), which has a relatively poorer prognosis has characteristic phenotype of CD1a(-), CD8(-), CD5(-) (dim), and positivity for 1 or more stem cell or myeloid antigens [9].

Acute Myeloid leukemias (AML), though relatively rarer than ALL in the pediatric age group, is usually diagnosed based on morphology and molecular and cytogenetic features. Flow cytometry, however, still has a role in sub-classification and in some instances may be extremely important for diagnosis, like acute myeloid leukemia with minimal differentiation as well as megakaryoblastic leukemias [10]. While ***Table* I** details various markers that are usually used for sub-typing AMLs, cytoplasmic myeloperoxidase is the most important marker that helps in assigning myeloid lineage.

Bi-phenotypic acute leukemia (BAL) is a very rare disease possibly arising from a hemopoietic pluripotent stem cells, are almost exclusively defined by Immunophenotyping. In fact, the scoring system proposed by the European Group for the Immunological classification of Leukemias (EGIL), was based solely on flow cytometric immunophenotyping, till World Health Organization (WHO) defined this entity as mixed phenotype acute leukemias (MPAL) in 2008. While the specificity for T-lymphoid and myeloid is based on cytoplasmic CD3 and cytoplasmic myeloperoxidase (MPO) antigens, respectively, the latter shown by either flow cytometry (FCM) or cytochemistry and/or clear evidence of monocytic differentiation, since there is no specific single antigen for B cells, lineage assignment here relies on the strong expression of CD19 together with another B cell-associated marker or, in cases with weak CD19, on the expression of at least 3 B-lineage markers [11]. ***Web Fig.* 3** shows few common acute leukemia phenotypes.

Lymphoma Diagnosis

Primarily based on morphologic assessment of the tissues involved with the disease is done with immunohistochemistry as an adjunct in the diagnostic algorithm. Diagnosis of lymphomas using flow cytometry has mainly been used for non-Hodgkin's lymphoma, though literature does describe its use in Hodgkin's lymphoma. Hitherto, flow cytometry was primarily used for diagnosis of lymphomas on fluid samples should they be available for analysis based on their involvement. In the pediatric age group, low grade lymphomas are extremely rare and the usual lymphomas seen include diffuse large B cell lymphoma, Burkitt's lymphoma and primary mediastinal large B cell lymphoma. T cell lymphomas, seen include hepato-splenic T cell lymphoma and rarely, peripheral T cell lymphoma (not otherwise specified). Utility of flow cytometry has been dealt with in the subsequent section on flow cytometry on lymph nodes.

Minimal Residual Disease (MRD) Detection Using Flow Cytometry in Pediatric Leukemias

Minimal residual disease (MRD) is defined as disease undetectable by morphologic examination. MRD is gaining importance nowadays both for therapy efficacy follow up and relapse risk estimation. Flow cytometric detection of MRD is based on identification of leukemic cells and their differentiation form normal, healthy cells by expressions of aberrant antigens or other phenotypic characteristics, called leukemia associated immuno-phenotypes (LAIPs). The advantages that FCM presents *vis-a-vis* other techniques is easy availability, rapid, convenient, and generally applicable technique for detecting MRD.

Dario Campana and Elane Coustan Smith were the pioneers in this field, especially in the detection of MRD in pediatric B cell ALLs. They not only demonstrated the effectiveness of flow cytometry in this area, but also helped in getting the therapy protocols based on the MRD analysis [12]. A cut-off of 0.01% and below is suggestive of the absence of the disease. Advances and upgradation of the technology in the area of flow cytometry including having additional lasers, newer antibodies, more fluorochromes and possibility of evaluation of more than 8 colors and adoption of bulk lysis method, the limit of detection has gone up significantly from 0.01% to now 0.001%. However, in centres with resource constraints, where 3-4-colour flow cytometers are still in use, MRD detection by 'MRD lite', using only 3 antibodies (CD19, CD34 and CD10) may be a useful tool to detect MRD in the bone marrow on Day 19 of induction chemotherapy, up to a detection limit of 0.01%. The MRD lite analysis is based on the fact that on Day 19, not >0.01% precursor B cells (hematogones) are present in the bone marrow. And thus presence of >0.01% CD19 positive B cells which co-express either CD34 and/or CD10 would be residual blasts, and not hematogones (which are also CD19+ with CD34 and/or CD10) [13,14]. This methodology has its disadvantages as (*a*) it cannot be used to evaluate MRD on Day 28-33 after completion of induction therapy which has better prognostication than MRD done on day 19; (*b*) not using LAIPs to differentiate residual blasts from precursor B cells may give false positive results, and (*c*) the limit of detection is 0.01%, which is one log lower than the one with advanced flow cytometry. ***Web Fig.* 4** shows scatter plots demonstrating the MRD in a case of B and T ALL.

FLOW CYTOMETRY ON LYMPH NODES

Lymphadenopathy is a common finding in children. Most of the times it is benign; however, it is a worrisome situation and needs diagnostic workup. The diagnosis of disease in a lymph node has always been based on morphology, be it a lymphoma, leukemia, metastasis or even reactive. Flow cytometry can provide a faster diagnosis with the same material provided for morphology, the material being an excised lymph node or fine-needle aspirate (FNA) from lymph node. While the morphology remains the cornerstone for diagnosis of hemato-lymphoid neoplasia, recent update of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (2016), has laid a significant emphasis on various mutations and genetic signatures. Despite this, immunophenotype continues to be an integral component for sub-classification, especially for lymphoid neoplasia. Immunophenotyping can be done by both, immuno-histochemistry (IHC) (done on tissue section) and flow cytometry (FCM) (done on cells extracted from tissue),

with FCM clearly scoring over IHC on may counts. Faster turnaround time, pick-up of immuno-phenotypically discrete cell populations, evaluating co-expression of various markers by multi-parameter FCM, quantitation of antigen expression based on mean/median fluorescence intensity and even grading of lymphomas by S-phase fraction analysis are some of the salient advantages of FCM *versus* IHC [15].

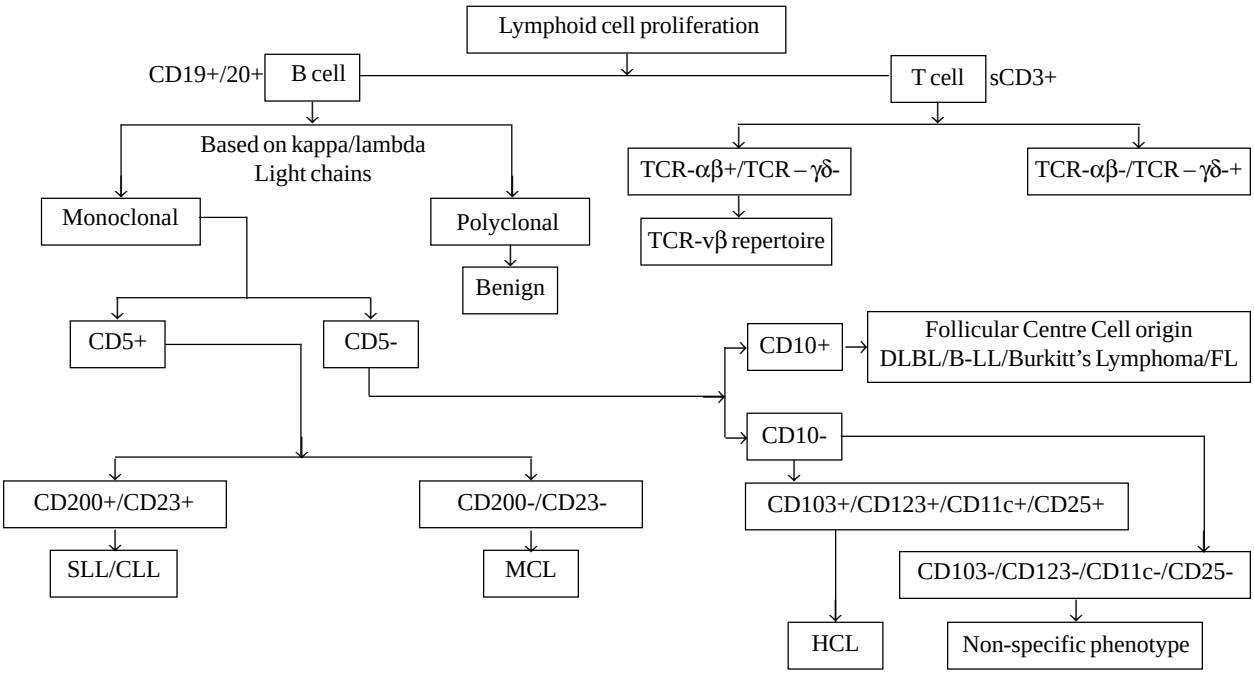
Lymphomas are the third most common childhood malignancies after acute leukemias and brain tumors, constituting 10-12% of childhood cancers [16]. Burkitts Lymphoma which is a high grade aggressive B cell Non Hodgkins Lymphoma (NHL) and considered to be a medical emergency, is the most common NHL in children and adolescents and accounts for around ~40% of NHL's in those under the age of twenty [17]. A faster diagnosis by flow cytometry in this situation can be life saving. Flow cytometry utilizes a panel of anibodies for immuno-phenotyping, based on which a diagnosis is made. It serves a variety of roles in the field of diagnosis including:

Acute Lymphoblastic Lymphoma: Flow cytometry is magical in picking up blastic population, by clearly separating abnormal blasts from the rest of the population using CD45. These abnormal blasts can then be further classified based on the array of antibodies used.

Example: 5-year-old boy presented with mediastinal mass. Tissue Biopsy done showed dim CD45 positive blasts, expressing cytoplasmic CD3, dim to negative CD3, very bright CD7, dim CD2, and dual negative CD4 and CD8, consistent with a diagnosis of T cell lymphoblastic lymphoma (***Web Fig.* 5**)

Lymphomas

(*a*) *Diagnosis*: Conventionally, diagnosis of lymphomas has been based on biopsy sections, both excised tissue as well as needle biopsies. Supplementation with IHC on these tissues has helped confirm, classify and sub-categorize, which in turn has ensured appropriate therapy protocols. While diagnosis has been made in majority of the patients based on the above mentioned procedures, few of the cases elude diagnosis for the fact that the involvement is subtle or have a considerable overlap with non-neoplastic entities. Immuno-phenotypic diagnosis for most of the hemato-lymphoid neoplasia requires an array of markers. Lymphomas, especially, non-Hodgkin's lymphoma, provide a unique opportunity to base the



Key: SLL: Small lymphocytic Lymphoma; CLL: Chronic lymphocytic Leukemia; MCL: Mantle Cell Lymphoma; HCL: Hairy Cell Leukemia; DLBL: Diffuse Large B Cell Lymphoma; B-LL : B-Lymphoblastic Lymphoma; FL: Follicular Lymphoma.

FIG. 2 *Algorithm for immunophenotypic classification of NHL.*

diagnosis on clonality assessment. The demonstration of clonality, for B-cells is done by using kappa and lambda light chains, best done on FCM since one is able to select discrete populations and look at the expression of light chains on them, unlike IHC, wherein a lot of background staining is noted on the sections, making it difficult for interpretation. T-cell clonality is relatively difficult both on tissue by IHC as well as by FCM and the diagnosis is primarily based on immuno-phenotypic aberrancies on the T cells. In addition, using antibodies against the variable region of T cell receptor antigen – beta (TCR-$\upsilon\beta$), clonal restriction to any of the 24 sub families of TCR-$\upsilon\beta$ can be assessed by FCM [15]. It also needs to be noted that in today's era of personalized medicine and targeted therapies, FCM helps in identification of various antigens for which targeted therapies are available, *e.g.* rituximab as anti CD20, alemtuzumab as anti CD52, daratumumab as anti CD38.

(*b*) *Classification*: Classifying lymphomas is needed to tailor therapy as per the immunophenotypic sub-groups. Application of a repertoire of antibodies helps in classification. While these can be applied on the tissue, FCM has a unique advantage of using minimal sample for applying them and easier interpretation since expression of various markers is ascertained on discrete cell populations. This becomes important when one is looking for expression of antigens not native to the population of interest [15]. For example, expression of CD5 (a T cell antigen) on B cells, *i.e.* CD5+ B cells. An algorithmic approach to immuno-phenotypic diagnosis of NHL is depicted in the ***Fig.* 2.**

(*c*) *Estimation of S-Phase Fraction*: Historically, morphological grading of lymphomas has been done morphologically and by Ki-67 immunostaining on the tissues. Morphologists have long been arguing on how, flow cytometry is in adept to provide this valuable information, which has both prognostic and therapeutic implications. However, the possibility of staining DNA and quantifying the same at various stages in cell cycle has made this easily available. Flow cytometry, helps in provision of reliable and more reproducible count of grading by 'S-Phase faction'. More importantly, the same is assessed on selected cell populations of interest [15]. An S-Phase fraction of <10% has been seen in low grade lymphomas, whereas >15% is seen in high grade lymphomas. An example of Burkitts lymphoma expressing CD19, CD20, CD10 and lambda restriction with High S-phase fraction is demonstrated in ***Web Fig.* 6**.

Flow cytometry, thus, is a valuable technique for immunophenotypic analysis of lymphomas, especially the NHLs. Researchers have also demonstrated its ability to characterize Hodgkin's Lymphoma as well. A few disadvantages, though, include requirement of fresh unfixed tissue and immediate processing as well as interpretative issues in some cases of T-cell rich B cell lymphoma.

DNA Ploidy and Cell Cycle Analysis

Flow cytometry is a rapid and reliable method for measuring nuclear DNA content [18]. Measurement of DNA content of individual cells helps provide information about their ploidy, which is of relevance in some neoplastic disorders like ALL, breast tumors, lymphomas, etc. DNA content of the cells is measured by staining with a fluorescent dye that binds to DNA ensuring reflection of an accurate amount of DNA present [18]. Propidium iodide (PI), is one of the most widely used dyes for such purposes. DRAQ5, FxCycle, bis-benzimadazole, Hoechst 33342, etc are other dyes used which help to observe a DNA histogram in viable cells without permeabilisation, unlike dyes like PI [18].

FLOW CYTOMETRY OF NON-HEMATOPOIETIC NEOPLASMS

Flow cytometry is not used routinely in the diagnosis or follow-up of non-hematopoietic neoplasms; however, many non-hematopoietic neoplasms and tissues are amenable to flow cytometric analysis, especially serous cavity effusions and limited fine-needle aspirate (FNA) or cerebrospinal fluid (CSF) samples [19]. It is possible to differentiate various non-hematopoietic malignancies with use of certain markers like EpCAM (Ber-EP4, CD326, and MOC31), CD45, CD56, CD71, CD81, CD9, MyoD1, Myogenin, CD99 and CD271. For instance, most carcinomas would be EpCAM+, CD45–, CD14–, while neuroblastomas would have the following profile: CD56hi, GD2+, CD81+, CD9+, CD45–. Rhabdomyo-sarcomas on the other hand would also express MYOD1+, myogenin+ [2,19]. In fact based on the following markers CD45, CD56, CD90, EpCAM, CD34, Myogenin and MyoD1, an algorithm for differentiating various small round cell tumours can be followed. Details of markers with their relative expressions is given in the ***Table* III**. An algorithmic approach to the diagnosis is also provided in the ***Fig.*3**. CD45 would clearly help differentiate hematopoietic (CD45+) versus non hematopoietic malignancies (CD45-). All the non-hemopoeitic malignancies can then be subdivided into three categories based on CD56 expression. A strong expression of CD56 is seen in Neuroblastoma, Wilms' tumour and Nasopharygngeal carcinoma. These can be further characterised based on the expressions of

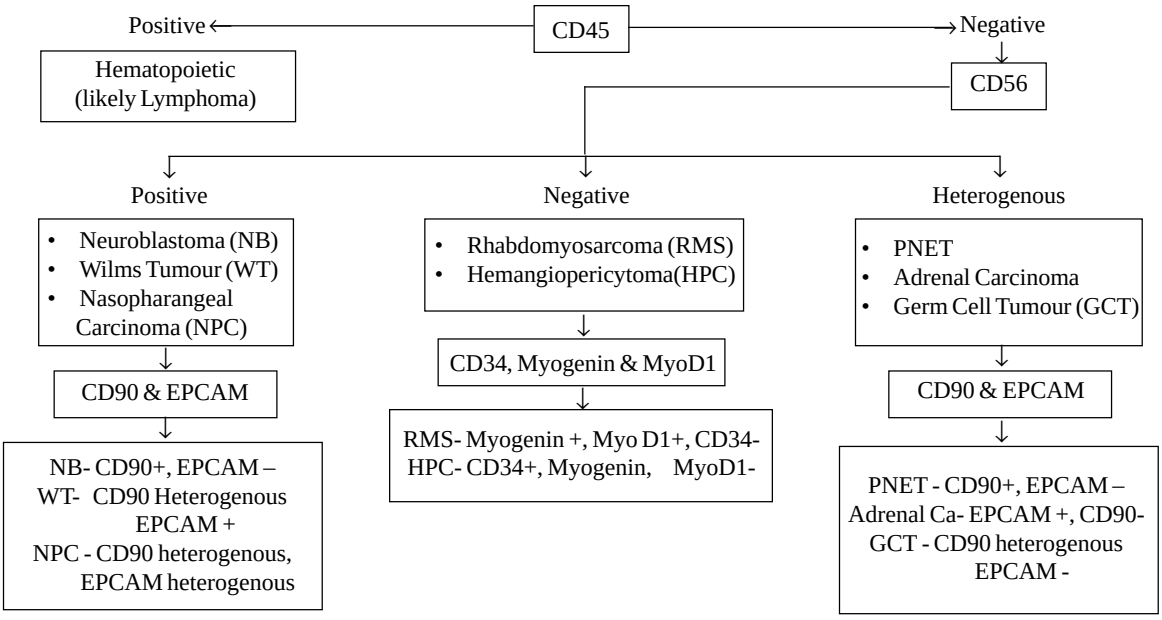


FIG. 3 *Algorithm for diagnosing small round cell tumor by flow cytometry.*

TABLE III IMMUNOPHENOTYPIC MARKERS FOR SMALL ROUND CELL TUMORS [2]

	CD56	*CD90*	*CD99*	*CD9*	*CD81*	*MyoD1*	*Myogenin*	*EPCAM*	*CD271*	*CD34*
Neuroblastoma	+	+	_	+	+	_	_	_	_	_
PNET	H	+	+	+	+	_	_	_	+	_
Rhabdomyosarcoma	_	+	_	H	H	+	+	_	_	_
Wilms tumour	+	H	_	H	H	_	_	H	H	_
Adrenal carcinoma	+	H	_	H	H	_	_	+	_	_
Nasopharyngeal carcinoma	+	H	_	H	H	_	_	+	_	_
Germ cell tumour	+	H	_	H	DIM +	_	_	_	_	_
Hemangiopericytoma	_	_	_	_	_	_	_	_	_	+

+ positive, – negative, H heterogeneous.

EpCAM and CD90. Neuroblastoma is CD90 positive and is negative for EpCAM-, Wilms tumour is strongly positive for EpCAM with a heterogenous expression of CD90, whereas nasopharygngeal carcinoma shows a heterogenous expression for both CD90 and EpCAM. Rhabdomyosarcoma and hemangiopericytoma are negative for CD56, and based on myogenin and myo-D1 positivity classified as rhabdomyosarcoma and CD34 positivity as hemangiopercytoma. A heterogenous expression of CD56 suggests the possibility of primitive neuroectodermal tumour (PNET), adrenal carcinoma or germ cell tumour (GCT). These can again be differentiated based on CD90 and EpCAM. PNET is CD90 positive and is negative for EpCAM-, while adrenal carcinoma has an inverse expression of these markers, being positive for EpCAM and negative for CD90. GCT is also EpCAM negative; however, it shows heterogenous expression for CD90. As is obvious from the above discussion, pediatric small round cell tumors, would clearly be amenable to accurate diagnosis using flow cytometry within a reasonably reduced time frame.

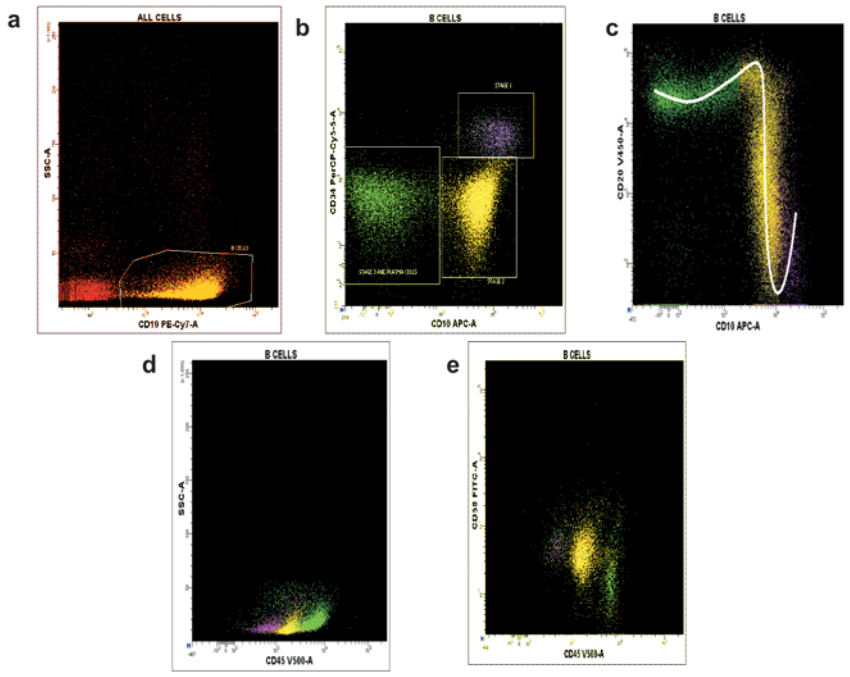
SUMMARY

Pediatric malignancies comprise a heterogenous group of disorders, which need a multi-modality approach for diagnosis. Flow cytometry is increasingly being

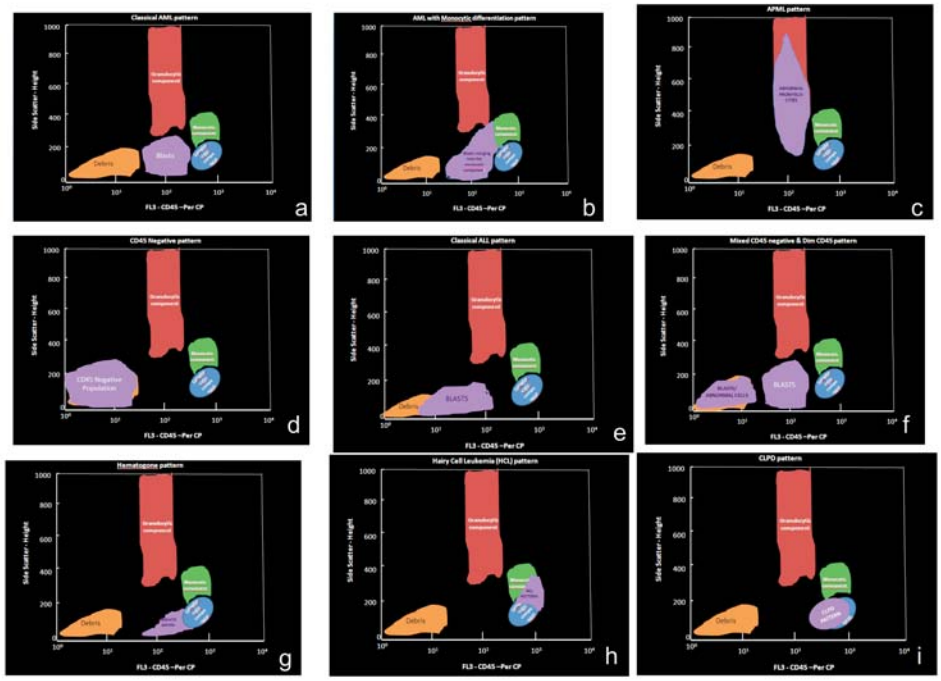
acknowledged as a valuable tool in the diagnostic algorithm. This is especially so in the hemato-oncological disorders like acute leukemias. Flow cytometry is also showing its imprint in grading and prognostication of various disorders, DNA ploidy and minimal residual disease detection being the prime examples of the same. Tissue samples and many non-hematopoietic malignancies are also increasingly subjected to flow cytometry for appropriate diagnosis and classification. Use of flow cytometry in conjunction with other adjunct modalities like molecular and cytogenetic studies is ensuring an accurate diagnostic and prognostication realm.

REFERENCES

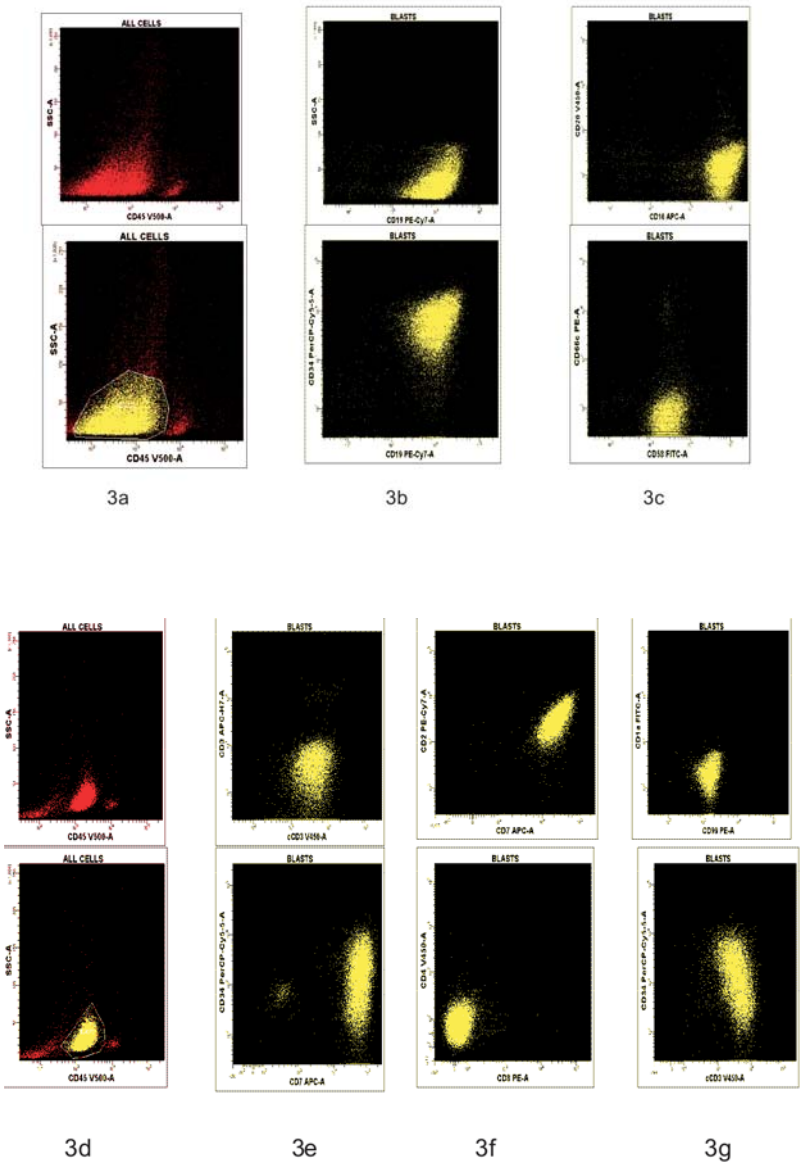
1. Arora RS, Eden TOB , Kapoor G. Epidemiology of childhood cancer in India. Indian J Cancer. 2009;46:264-73.
2. Ferreira-Facio CS, Milito C, Botafogo V, Fontana M, Thiago LS, Oliveria E, *et al*. Contribution of multiparameter flow cytometry immunophenotyping to the diagnostic screening and classification of pediatric cancer. PLoS ONE. 2013;8: e55534.
3. Pillai V, Dorfman DM. Flow cytometry of non-hematopoietic neoplasms. Acta Cytologica. 2016;60:336-43.
4. Li J, Wertheim G, Paessler M, Pillai V. Flow Cytometry in pediatric hematopoietic malignancies. Clin Lab Med. 2017;37:879-93.
5. Arnoulet C, Be´ne´ MC, Durrieu F, Feuillard J, Fossat C, Husson B, *et al*. Four- and five-color flow cytometry analysis of leukocyte differentiation pathways in normal bone marrow: A reference document based on a systematic approach by the GTLLF and GEIL. Cytometry Part B:Clin Cytometry. 2010;78B:3-10.
6. Mckenna RW, Asplund SL, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) and neoplastic lymphoblasts by 4-color flow cytometry. Leuk Lymphoma. 2004;45:277-85.
7. Handoo A, Dadu T, Rangan A, Bachchas V, Sartor M, Choudhary DR, *et al*. CD45-side scatter dot plot in malignant hematological flow cytometry – scanner view for prediction of immunophenotypic diagnosis/lineage assignment and an optimizing tool for cost effective flow cytometry. Int J Lab Hemat. 2012;34:50 (Abstract).
8. Wang XM. Advances and issues in flow cytometric detection of immunophenotypic changes and genomic rearrangements in acute pediatric leukemia. Transl Pediatr. 2014;3:149-55.
9. Jain N, Lamb AV, O'Brien S, Ravandi F, Konopleva M, Jabbour E, *et al*. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. Blood. 2016;127:1863-9.
10. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood. 2008;111:3941-67.
11. Borowitz MJ, Bene MC, Harris NL, Porwit A, Matutes E. Acute leukemias of ambiguous lineage. *In*: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J Vardiman JW, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008. p. 150-5.
12. Campana D, Coustan-Smith E. Detection of minimal residual disease in acute leukemia by flow cytometry. Cytometry. 1999;38:139-52.
13. Coustan-Smith E, Ribeiro RC, Stow P, Zhou Y, Pui CH, Rivera GK, *et al*. A simplified flow cytometric assay identifies children with acute lymphoblastic leukemia who have a superior clinical outcome. Blood. 2006;108:97-102.
14. Chatterjee T, Somasundaram V. Flow cytometric detection of minimal residual disease in B-Lineage acute lymphoblastic leukemia by using "MRD lite" panel. Med J Armed Forces India. 2017;73:54-7.
15. Braylan RC. Impact of flow cytometry on diagnosis and characterization of lymphomas, chronic lymphoproliferative disorders and plasma cell neoplasias. Cytometry A. 2004;58:57-61.
16. Consensus Document for Management of Pediatric Lymphomas and Solid Tumors – Prepared as an outcome of the ICMR-sub-committee on paediatric lymphomas and solid tumors. ICMR 2017:29. Available at URL: *http://www.icmr.nic.in/guide/cancer/PEDIATRIC%20LYMPHOMAS%20AND%20SOLID%20TUMORS%20final%20pdf.pdf*. Accessed October 09, 2017.
17. Miles RR, Arnold S, Cairo MS. Risk factors and treatment of childhood and adolescent Burkitt lymphoma/leukaemia. Br J Haematol. 2012;156:730-43.
18. Darzynkiewicz Z, Halicka HD, Zhao H. Analysis of cellular DNA content by flow and laser scanning cytometry. Adv Exp Med Biol. 2010;676:137-47.
19. Pillai V, Dorfman DM. Flow cytometry of nonhematopoietic neoplasms. Acta Cytologica. 2016;60: 336-43.



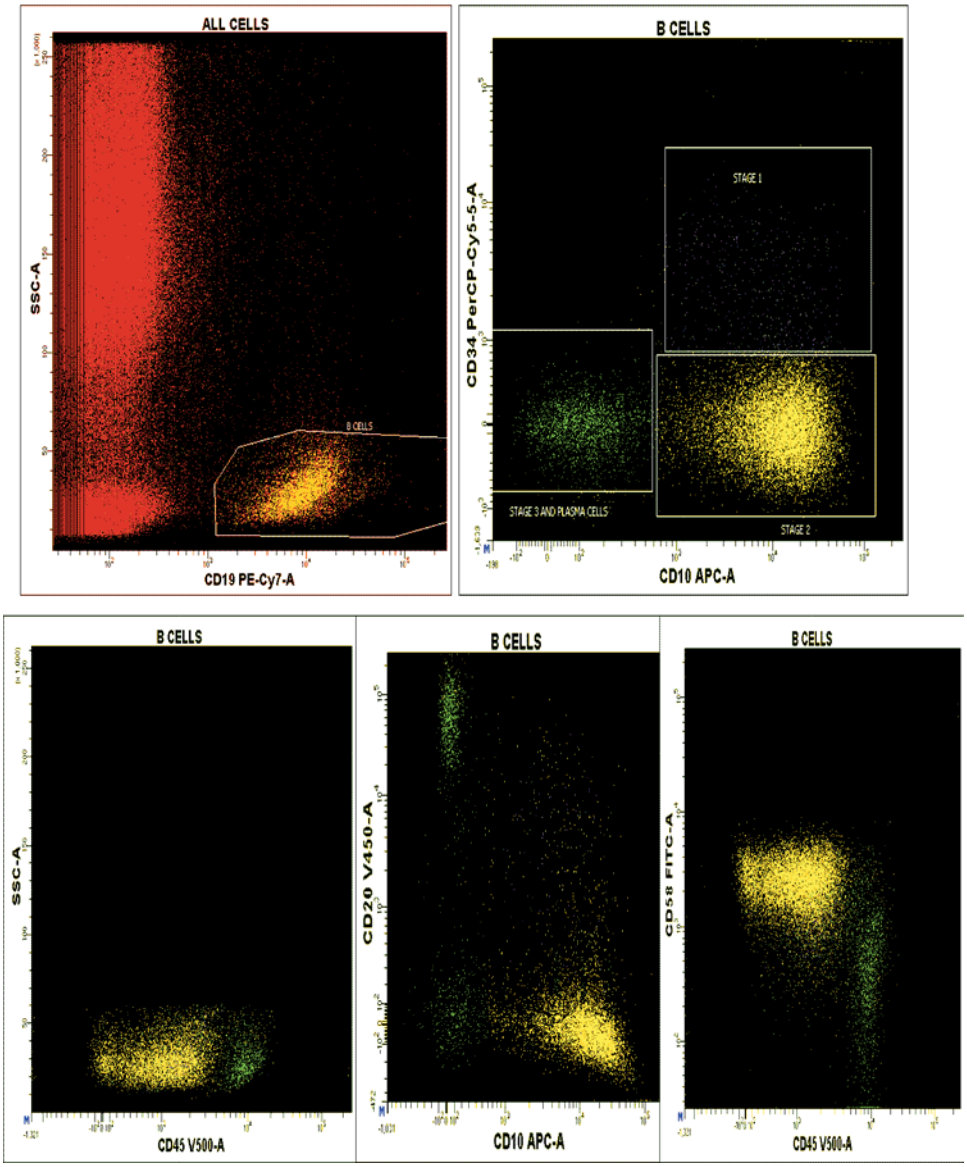
WEB FIG. 1 *Show typical phenotypic patterns of hematogones; a: shows CD19 based gating strategy; b: Shows sub gating based on CD34 and CD10; c: Shows classical waterfall pattern; d: shows the merging pattern on CD45/SS; e: CD58vsCD45, showing maturation pattern.*



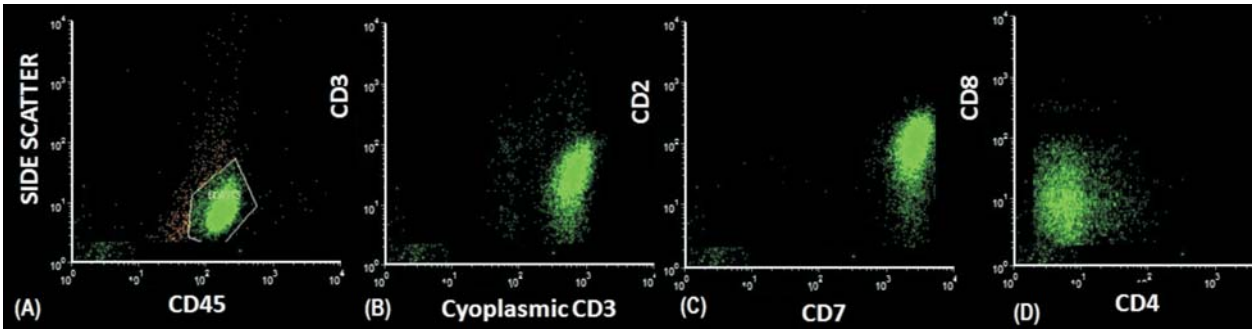
WEB FIG. 2 *Location of blasts and/or abnormal cells.*



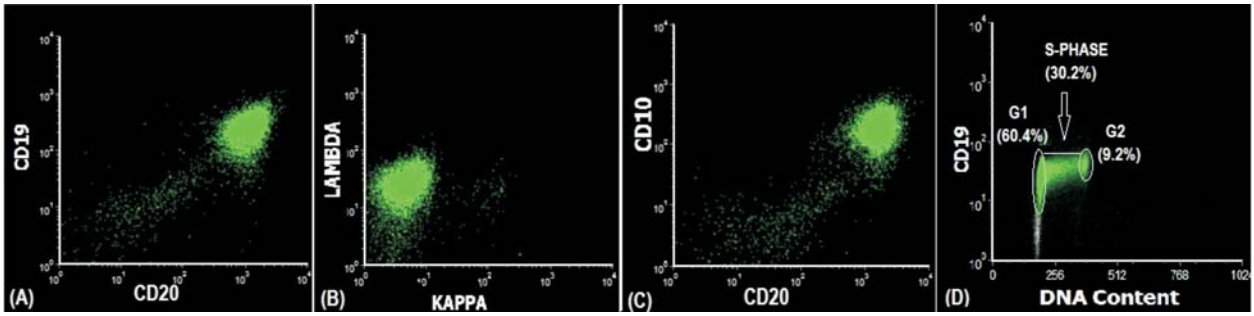
***Web Fig.* 3** (a-c) *Patterns in Precursor B cell ALL, (d-g): Patterns of Precursor T cell ALL.*



WEB FIG. 4 *Minimal residual disease patterns in Precursor B Cell ALL.*



Web Fig. 5 *(A) Cells expressing CD45, suggestive of blasts (B) Blasts express cytoplasmic CD3 with dim to negative surface CD3, suggesting T cell Lymphoblastic Lymphoma (C&D) T blasts express very bright CD7 and dim CD2, and are negative for CD4 and CD8.*



WEB FIG. 6 *Cells expressing CD19, CD20 show lambda restriction, suggestive of B cell Non Hodgkins lymphoma (C) Lambda restricted B cells show CD10, consistent with CD10 Positive B cell Lymphoma (D) High S-phase fraction on lambda restricted B cells, suggesting Burkitts Lymphoma.*