Flow Cytometry in Haematolymphoid Neoplasms - Principle and Applications

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ABSTRACT

BACKGROUND

The criteria for identification of distinct disease entities among the haematological malignancies has been immensely improvised during the past twenty years due to the knowledge of immunophenotyping and cytogenetic studies, which are being used by the World Health Organization (WHO) for classifying the hematopoietic and lymphoid tissues. It endorses a multiparametric approach for the diagnosis of different distinct entities and has taken into account not only the morphologic and cytochemical features but also immunophenotypic, cytogenetic as well as molecular characteristics of the various disorders. Immunophenotyping has a great role to play in defining as well as classifying acute leukaemias. Similarly flow cytometric immunophenotyping has become an integral part of investigation of mature lymphoid neoplasms in clinical practice, precisely in the diagnosis, classification, staging and monitoring of treatment response. Needless to say that immunophenotyping has a crucial role in characterizing and distinguishing plasma cell neoplasms and by allowing detection of maturation asynchrony and aberrant antigen expression on multiple bone marrow lineages, it helps in identifying and characterizing the myelodysplastic group of disorders, thus immunophenotypic analysis is of immense help in today's perspective for studying and evaluating all and every aspect of haematolymphoid malignancies. This review presents the principle and applications of flow cytometry (FC) with respect to haematolymphoid neoplasms.

KEYWORDS

Haematological Malignancies, Immunophenotyping, Flow Cytometry, Lineage Specific Markers, Cluster Differentiation (CD) Molecules

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BACKGROUND

Over the period of last 20 years, knowledge of immunophenotyping and cytogenetic studies has improvised the criteria for identification of distinct disease entities among the haematological malignancies.[1] The World Health Organization (WHO) classification for tumours of the hematopoietic and lymphoid tissue endorses a multiparametric approach for the diagnosis of different distinct entities. It has taken into account not only the morphologic and cytochemical features but also immunophenotypic, cytogenetic as well as molecular characteristics of the various disorders.[1]

Flow cytometric analysis is ideal in fluids, where cells are naturally suspended.[2] It is also useful in the analysis of solid tissues, from which single-cell suspensions can be obtained. This precisely allows diagnosis and subtyping of hematologic tumours as defined by WHO. Moreover, flow cytometry immunophenotype (FCI) can accurately detect the tumour cells as low as 1 in 10 and thus provides high sensitivity for minimal residual disease detection that is proved to have significant impact in management of hematologic neoplasms.[3] In addition, its role is crucial in management of hematologic neoplasms as apart from immunophenotypic characterization, it provides accurate quantitation of tumour cells. Hence FC has become an integral part of haematology laboratories. In this article, we will discuss the general principal flow cytometry and its application in diagnosis, classification, monitoring and treatment of hematolymphoid neoplasms (HLN). Table 1 enlists clinical applications of flow cytometry in haematology.

General Principles

Flow cytometry (FC), literally meaning measurement (-metry) of characteristics of cells (cyto -) in flow, is a powerful tool that illustrates simultaneously multiple proteins (antigens) expressed on the surface and / or intracellular compartments (cytoplasm and nucleus) of an individual cell as they flow in a fluid stream through a beam of light. FC measures optical and fluorescence characteristics of single cells (or any other particle, including nuclei, microorganisms, chromosome preparations, and latex beads). Physical properties, such as size (represented by forward angle light scatter) and internal complexity (represented by right - angle scatter) can resolve certain cell populations. This can be achieved in two ways. One way is that the different cellular components such as DNA or RNA can be bound or intercalated to fluorescent dyes. Other way is that; these fluorescent dyes can be conjugated to different antibodies which in turn can bind to the specific proteins on cell membranes or inside cells. When labelled cells are passed by a light source, the fluorescent molecules are excited to a higher energy state. Upon returning to their resting states, the fluorochromes emit light energy at higher wavelengths. The use of multiple fluorochromes, each with similar excitation wavelengths and different emission wavelengths (or "colours"), allows several cell properties to be measured simultaneously. Commonly used dyes include fluorescein isothiocyanate (FITC), phycoerythrin (PE), and allophycocynin (APC) along with several others in the market. Even longer wavelengths and more colour scans can be created by usage of Tandem dyes having internal fluorescence resonance energy transfer.

Inside, a flow cytometer, cells are made into suspension to be drawn into a stream.[4] This is created by surrounding sheath of isotonic fluid which creates laminar flow, which allows the cells to pass individually through an interrogation point. Usually, a laser is used to produce beam of monochromatic light that intersects the cells in the laminar flow at the point of interrogation. Emitted light, given off in all directions is collected via optics. Optics direct the light to a series of filters and dichroic mirrors that isolate particular wavelength bands. The light signals are detected by photomultiplier tubes and digitized for computer analysis.[5] Figure - 1 represents the fluidic and optical components of a flow cytometer. The resulting information usually is displayed in histogram or two dimensional dot plot formats using specially designed software.

ROLE OF IMMUNOPHENOTYPING IN THE EVALUATION OF HEMATOLOGIC NEOPLASMS

As defined by WHO haematolymphoid neoplasms are broadly categorized on the basis of their lineages like myeloid, B cell and T cell malignancies. Further, they are divided into precursor i.e. blastic and peripheral i.e. mature neoplasms. Based on the cytomorphology, immunophenotyping characteristics and cytogenetics analysis, these neoplasms are further subclassified into different specific disorders.[5]

Immunophenotyping in Acute Leukaemia

It has a great role in defining as well as classifying acute leukaemia as many a times. It is not possible to specify the blasts into myeloid or lymphoid lineage by evaluating their morphology or cytochemical staining characteristics.[6] In such cases use of a comprehensive panel including intracytoplasmic lineage specific markers is indicated. Table 2 shows different markers expressed by myeloid, B cell and T cell lymphoid lineages including lineage specific markers (indicated with *).[7] Using this information, FCI is applied to diagnosis, lineage identification and further sub-classification.

Acute Lymphoblastic Leukemia (ALL)

Table 3 shows immunophenotypic classification of B - cell and Figure - 2 shows a typical example of immunophenotypic analysis of B - cell ALL. CD10 antigen is expressed by both B ALL and T ALL hence it is also known as "common ALL antigen" (CALLA). Its positivity is also seen in two thirds of the ALL patients. Positivity of morphologically - appearing lymphoid - lineage cells for the terminal deoxynucleotidyl transferase enzyme (nTdT)

supports their phenotypic immaturity.[8] While studying B cell ALL, it is important to know antigen expression pattern of normal B cell precursors i.e. hematogones. Hematogones are present in bone marrow of all normal individuals; however, in children they are present in high percentages. In children with immune thrombocytopenia (ITP) or viral infection with pancytopenia, hematogones may increase in bone marrow giving false impression of B ALL and hence it is very important to know patterns of antigen expression by hematogones which is shown in Table - 4. As hematogones are results of the normal sequence of antigenic evolution in B - cell precursors, they exhibit typical complex spectrum of antigenic expression and lacks any kind of aberrant expression. Whereas in precursor B - ALL, lymphoblasts undergo maturation arrest which leads to several aberrancies like expression of CD13, CD33, etc. which can be detected immunophenotypically.[9]

Expression by an early B cell precursors are surface intermediate CD34, weak CD22, strong CD10, and weak CD19 along with nTdt, and cytoplasmic CD79a (cCD79a). Immediately after, the B - cell precursors sequentially start losing CD34 and nTdT, decrease CD10 expression, and display reactivity for CD20 (Table 4). B-cell precursors, later on, start producing Ig heavy chains which gets accumulated in the cytoplasm until Ig light chains are produced. This leads to IgM molecules expression over the cell surface of a functionally immature B - lymphocyte. Precursor B - ALL patients were classified into three major groups on the basis of maturation sequence of the normal BM B - cells, (Table 3); however, this classification is no more used as it is of little clinical significance. Instead, current WHO 2008 classification is based on cytogenetic abnormality as it predicts prognosis and treatment response. Some immunophenotypic patterns of antigen expression are shown to be associated with underlying cytogenetic abnormalities. Expression of CD25 along with that of myeloid markers like CD13 or CD33 is reported to be associated with t (9 ; 22), while lack of CD10 and CD24 expression in combination with expression of CD15 is found to be associated with t (v5 ; 11q23) ; MLL gene rearrangement, a prognostically unfavourable gene rearrangement that is commonly seen in infants.[10] In ALL with t (12 ; 21) (p13 ; q22) a prognostically favourable abnormality, the blasts show bright expression of CD10 with dim / absent CD9, CD20 and CD66c.[11]

T - cell lymphoblastic lymphoma most often shares immunophenotypic characteristics corresponding to that of common thymocyte stage of ALL; it shows much more variability in expression patterns of CD4 / CD8 expression, significant T - cell antigen deletion, and absence of the CD3 and variable TdT expression (Figure 4). T ALL is currently divided into four groups (Table 4): pro - T (or TI), pre - T (or TII), cortical or TIII), and mature (or TIV) ALL. Pro - T ALL typically exhibits co expression of two early T - cell markers - CD7 and cCD3 while lacking expression of other T – cell - associated antigens. Pre - T ALL cases show surface CD2, CD5, and / or CD8 in addition to CD7 and cCD3. Similar to cortical thymocytes, leukemic cells from cortical T-ALL also display CD1a reactivity.

Patients with T - lymphoblastic lymphomas more often express TIV / mature T-ALL phenotype (sCD3, CD1a, CD4, or CD8) rather than a pure T-ALL. In both TIII and TIV T-ALL, surface expression of CD3 may be associated with expression of TCR of either the TCR α / β or TCR γ / δ type.[12] This classification is also not much useful for prediction of prognosis and treatment response.

Recently, a new type of T-ALL 11 has been defined i.e. "Early T Cell Precursor ALL (ETPALL)" which is shown to have poor prognosis and poor treatment response to current therapy. Blasts in ETPALL defined with characteristic immunophenotype i.e. they show absence of CD1a and CD8 expression, weak CD5 expression and expression of one or more myeloid - associated (CD117, CD33, CD13) and / or stem cell - associated 12 markers (Cd34).

Acute Myeloid Leukemia (AML)

FCI has its usefulness in order to differentiate acute myeloid leukemias from acute lymphoid leukemias so as to determine the appropriate management; accordingly predict the prognostic outcome; and finally, in evaluating relapse / residual disease by appropriately defining a blast immunophenotype. Though CD117 together with CD13 and CD33 is one of the earliest antigens that can be detected as soon as hematopoietic precursors are differentiated into myeloid cells; yet when individually considered, none of these markers specify myeloid lineage of leukemic cells. Amongst all myeloid antigens, demonstration of cytoplasmic MPO is considered to define the lineage. In detection of residual or relapsing disease; an aberrant expression of AML immunophenotype showing CD7, CD19, CD56 etc. is quite useful and thus helps in distinguishing leukemic from recovery blasts (Figure 5). In regards to classification of AML, FCI reveals some typical marker expression which helps in subclassification in AML and further in prediction of underlying cytogenetic abnormality. For example, AML M2 commonly shows expression of CD19 in addition to myelomonocytic markers (i.e., CD13, CD33) which is characteristically associated with t (8; 21); the myeloblasts in this type of leukaemia also typically express CD34 as well as CD56. Expression of CD56 indicates poor prognosis. Acute promyelocytic leukemia (APL; AML - M3) characteristically lacks the expression of CD34 and HLA-DR. However, expression of HLA - DR and / or CD34 can be seen in rare cases of APL. Markers like CD11b, CD11c, CD14, CD15, CD33, CD64 are commonly associated with monocytic differentiation and particularly CD14 is a monocyte - specific marker. However, these markers may be diminished or absent in AML with monocytic differentiation (AMML, M4 and AMOL, M5). Thus, it has become crucial while diagnosing AMLs with monocytic differentiation to correlate the immunophenotypic markers with nonspecific esterase staining, especially alpha naphthyl acetate esterase (ANAE). CD235a and CD71 helps in categorization of erythroleukemia (AML M6) and similarly CD41 and CD61 in megakaryoblastic leukemia (AML M7).

Mixed Phenotypic (Bi-Phenotypic) Acute Leukemia (MPAL)

In the recent edition of the WHO classification of tumours of hematopoietic and lymphoid tissues,[13] the group of acute leukemia where blast cells exhibit features from more than one lineage has been defined as mixed phenotypic or bi - phenotypic acute leukemia (MPAL / BAL). MPAL has been partitioned according to the lineage mix they display, i.e., as B / myeloid, T / myeloid and rare types including triple lineage or B / T co - expression.

This group includes both type of cases i.e., bilineal cases where two types of blasts of different lineage co-exist and truly aberrant cells co expressing normally exclusive markers. According to recent WHO, myeloid lineage is defined by the expression of myeloperoxidase or, for cells already showing differentiation towards the monocytic lineage, by the expression of at least two of the monocyte associated antigens CD11c, CD14, CD64, lysozyme and / or non-specific esterase activity demonstrated in cytochemistry. Similarly, cytoplasmic or surface (rare) expression of the epsilon chain of CD3 are specific for T lineage committed cells. Several markers are required in order to specify B - lineage engagement i.e., strong CD 19 expression along with concomitant expression of at least one of the following: cytoplasmic or membrane expression of CD79a, CD22, and / or CD10 surface labelling, is required. Moreover, in case of weak CD 19 expression, CD22 and CD10 surface labelling, must be present to confirm B - lineage features.[14]

Immunophenotyping in Mature Lymphoid Neoplasms i.e. Chronic Lymphoproliferative Disorders (CLPDs)

Flow cytometric immunophenotyping is an integral part of investigation of mature lymphoid neoplasms in clinical practice, precisely in the diagnosis, classification, staging and monitoring of treatment response.[15] In addition to lineage assignment, FCI plays a significant role in B - cell non-Hodgkin's lymphoma and chronic B - cell leukemia by determining clonality that is immunoglobulin (Ig) light-chain restriction defining the size of cells,[16] for example high FSC reflects large size of cells, simultaneous evaluation of aberrant antigen expression and abnormal levels of routine antigen expression.[17] Mature B - cell neoplasms commonly express pan - B cell markers like CD 19, CD20, CD22 and CD79b ; additionally they also express markers indicating their source of origin like CD5, CD 10. They are clonal expansion of B - cells derived from a single abnormal B - cell that in majority of cases has evidence of Ig gene rearrangement.[18] In normal or reactive B - cell population, the ratio of surface kappa light chain expressing B - cells to surface lambda light chain expressing B - cells is 4:1 to 1:1 and a deviation from this range alerts underlying monoclonal B - cell proliferation.[19] Thus, any B cell population is defined as a clonal population when it is illustrated to express predominantly one type of surface Ig light chain that is either kappa or lambda.[20] Expression of certain aberrant antigens is considered to be surrogate markers in sub-classification of mature B-cell neoplasms.

Figure 6 demonstrates the approach to sub classification of B cell CLPDs using immunophenotyping and Table 5 shows typical immunophenotype of common B CLPDs. In contrast, for T cell CLPD, it is difficult to establish clonality by FCI and need a comprehensive panel of antibodies against variable region of beta chains of T cell receptors i.e. TCR V beta repertoire. However, in many cases, they show aberrant loss of pan - T cell markers i.e. CD7, CD3, CD5, CD2 and show CD4 + or CD8 + subset restriction. This helps in diagnosis and further classification in T cell CDPD. Using CD4 and CD8 expression T CLPDs can be further categorized into four groups (See Figure 7).

Immunophenotyping in Plasma Cell Neoplasms

Immunophenotyping has a crucial role in characterizing and distinguishing normal plasma cells from that of the neoplastic ones. It is based on the degree of expression of surface antigens, display of aberrant antigens and detection of intracytoplasmic immunoglobulin.[6] Normal plasma cells display intense expression of CD38 along with co-expression of CD138. Low levels of CD45 and CD19 is also a characteristic finding pertaining to normal plasma cell expression. Polyclonal intracytoplasmic immunoglobulin light chain and absence of surface immunoglobulin and common surface B - cell markers (CD20, CD22) also signifies the normal plasma cell expression characteristics. On the other hand, neoplastic plasma cells are characterized by expression of monoclonal cytoplasmic immunoglobulin, aberrant antigens such as CD56 or CD 117, diminished CD38 and / or CD138, and complete absence of CD19 and / or CD45. FCM monitoring in multiple myelomas is also very useful in predicting disease outcome after transplant. It is estimated by the proportion and recovery of normal versus neoplastic plasma cells.[6] Assessment of circulating myeloma cells in peripheral blood has a prognostic significance. FCM measurement of the S - phase fraction in order to determine the plasma cell proliferation rate, is a valuable tool in evaluating disease status and prognosis in myeloma patients. Moreover, FCI MRD evaluation in myeloma has also shown to be independent prognostic factor in the management if multiple myeloma.[21] Thus, FCI is quite a useful tool in diagnosis as well as prognosis and monitoring of plasma cell neoplasms.

Role of Flow Cytometry in Minimal Residual Disease Monitoring

Minimal residual disease (MRD) refers to a remaining neoplastic cell population that can be detected only by laboratory techniques more sensitive than morphology, like FC or polymerase chain reaction. It is a strong indicator of chances of relapse in childhood and adult leukemia, irrespective of the methodology used.[22] It distinguishes well the MRD - negative patients who responded well to therapy and therefore who should be spared from further therapy and distinguishes them from patients with residual tumours, that is MRD - positive patients, who need

continuation or even intensification of therapy. Thus, it guides in therapeutic decisions. FCI detects aberrant immunophenotype which is not characteristic of normal precursor cells or mature cells. Flow can successfully detect and quantify MRD level up to one leukemic cell in 10,000 normal cells and thus can be widely used for monitoring not only acute leukaemia but also mature lymphoid neoplasms such as adult T - cell leukaemia / lymphoma, small lymphocytic lymphoma, hairy cell leukaemia, and multiple myeloma.[23] It is rapid to perform, simple and provides quantitative data as well as adequately sensitive. Hence FC monitoring of MRD is widely used method.

Immunophenotyping in Myelodysplastic Syndrome (MDS)

FCI allows detection of maturation asynchrony and aberrant antigen expression on multiple bone marrow lineages even in the absence of overt morphologic dysplasia and increased blast percentages. FCI illustrates dysplastic maturation of both myeloid and erythroid lineages by comparing deviation patterns of antigen expression on a dysplastic cell population with identification on normal cells. In 2006, an international working conference on MDS included FC evaluation of bone marrow cells as a co-criterion in the proposed minimal diagnostic criteria's also useful in immunophenotyping MDS,[24] because it allows for the detection of an accurate percentage of myeloblasts; microblasts are characteristic of MDS and often difficult to morphologically differentiate from lymphocytes. Normal hematopoietic cells express antigens in consistent manner and show reproducible patterns with maturation. In contrast, clonal dyspoietic cells in MDS shows increased or decreased intensity and / or loss of normal antigens, asynchronous maturational expression, aberrant expression of lymphoid antigens and abnormal, low systemic sclerosis (SSC) in granulocytes due to hypo granularity.

Immunophenotyping in Other Myeloid Disorders

In more than 90 % of myeloid disorders other than chronic myeloid leukemia,[25] identification of abnormal myeloid populations can be done by using 4 - colour flow cytometry which supports its crucial role in diagnosing the myeloproliferative disorders (MPDs) and MDSs having clonal cytogenetic abnormalities.[26] A study done by Kussick and Wood showed one of the most useful combinations for analysing these myeloid markers which included the following : HLADR and CD33, and CD11b and CD16 or CD13 and CD16. Flow cytometric immunophenotyping is quite helpful in diagnosis of chronic myelomonocytic leukemia (CMML).[27] It can be done because of the accuracy in detecting the percentage of monocytic cell population through CD14 and CD64 analysis. Moreover, FCI can be quite useful in revealing abnormalities like the partial loss of CD13, CD14, and CD15 and expression of CD56 in morphologically mature monocytes of CMML that are not observed in normal hematopoietic cells.[28]

Flow cytometry also contributes too many clinical laboratory practices as mentioned in Table 1. It is not possible to cover these applications of flow cytometry in detail due to the limited scope of this article.[29] So to conclude, flow cytometry has gained immense popularity in the last two decades and has become an integral part of all research laboratories and it is a must for any laboratory dealing with management of haematolymphoid neoplasms. Its major advantage is rapid and simultaneous determination of multiple antigens leading to accurate and objective results. It plays an invaluable role in the investigation of haematolymphoid neoplasms by determining their lineage, classification and prognostic factors and by helping in monitoring therapeutic responses.

In addition, it contributes immensely to the understanding of biology of haematological diseases. Stringent coupled with quality control of instrumentation, reagents and procedures, continual staff training and participation of the staff in a proficiency testing program are essential components of this rapidly evolving technology.[30]

Immunology	• Immunodeficiency studies (CD4 + / CD8 + T cell enumeration) • Histocompatibility cross - matching • Transplantation rejection • HLA - B27 detection
Oncology	• DNA content and S phase of tumours • Measurement of proliferation markers like Ki - 67, PCNAa
Haematology	• Leukaemia and lymphoma phenotyping Identification of prognostically important subgroups • MRD detection • Diagnosis of MDS • Hematopoietic progenitor cell enumeration (CD34 + enumeration) • Diagnosis of systemic mastocytosis • Reticulocyte enumeration • Autoimmune and alloimmune disorders • Immune complexes Complement, IgG • Feto - maternal haemorrhage quantification
Blood banking	• Immunohematology • Assessment of leukocyte contamination of blood products
Genetic disorders	• PNH • Leukocyte adhesion deficiency

Table 1. Clinical Applications of Flow Cytometry

Blast	Weak CD45, CD34, HLADR
Myeloblasts	*MPO, CD117, CD13, CD33, CD15, CD1lb
Monoblasts	CD14, CD13, CD11b, CD11c, CD33, CD36, CD64, CD65, CD66,
Lymphoblasts	CD 19,* cyto / sCD22, cytoCD79a, CD 10, nTdT
T Lymphoblasts	*cyto / sCD3, CD1a, CD2, CD4, CD5, CD7, CD8, CD10, nTdT
Granulocytes	CD13,CD11b, CD11c,CD15,CD16,CD33,CD66,CD10
Monocytes	CD4, CD14, CD13, CD11b, CD15, CD33, CD36, CD64, CD68, CD 123, HLADR, bCD45
Mature B cells	CD19, CD20, CD22, CD79b, FMC7, HLADR, Kappa, Lambda32, bCD45
Mature T cells	CD2, CD3, CD4, CD5, CD7, CD8, strong CD45
NK cells	CD2, cytoCD3, CD733, vCD8, CD16, CD56, CD57, CD94, CD158, bCD45
Plasma cells	CD138, bCD38, CD19, bCD27, cyto79a, cyto - Kappa, cyto - Lambda
Erythroid blasts	CD235a, CD10, CD36, CD71
Megakaryoblasts	CD41, CD42, CD61

Table 2. Lineage Associated Antigens on Different Hematopoietic Cells

Note Abbreviations* these are lineage specific markers, b; bright, Cyto; cytoplasmic, s; surface

B - ALL Subtypes	Common Phenotypes
B - precursor ALL or Pro BALL	DR, CD19, cyto CD22, cyto CD79a, Cd34, TdT
B - ALL (CALLA +)	DR, CD19, CD20 + / -, cyto CD22, cyto CD79a, CD 10, CD34 (-), TdT (-), slg
Pre - B ALL	DR, CD 19, CD20 + / -, cyto / sCD22, cyto CD79a, CD10, CD34 (-), cIgM (cyto i.t), TdT + / -

Table 3. Immunophenotypes of B - All Subtypes

Antigens	Early (Stage 1)	Intermediate (Stage 2 & 3)	Mature B cells
TdT	+	-	-
CD34	+	-	-
CD10	Strong	Weak	-
CD19	Weak	Intermediate	Strong
CD22	Weak	Weak	Intermediate
CD20	-	(- / +) weak	Intermediate
CD38, 34	Strong	Strong	Variable
CD45	Weak	Intermediate	Strong
CD58	Weak	Weak	Weak
CD81	Strong	Strong	Intermediate
Cyt IgM	-	+	+
sIg light chains	-	- 1 +	+

Table 4. Variation in the Levels of Antigen Expressions in Different Stages of Normal Differentiating B Cell Precursors (Hematogones)

Abbreviations: TdT, terminal deoxynucleotidyl transferase; cyt IgM, cytoplasmic IgM; sIg, surface immunoglobulin. (+) ; positive, (-) ; negative, (- / +) spectrum from negative to weak positive

Disorder	Common Phenotypes
Chronic Lymphocytic Leukemia	DR, CD 19, CD20, CD5, CD22 (-), CD23, CD10 (-), CD11c + / -, CD25 + / -, CD43, weak sIgM and sIgD
Pro - Lymphocytic Leukemia	DR, CD 19, CD20, CD5 (-), CD22, CD23 (-), CDl0 (-), bright sIg
Mantle cell Lymphoma	DR, CD19, CD20, CD22, CD5, CD23 (-), CD10 (-), CD43
Follicular Cell Lymphoma	DR, CD 19, CD20, CD22, CD5 (-), CD23 + / -, CD10, CD11c (-), CD43 (-), bright sIg
Hairy Cell Leukemia	DR, CD19, CD20, CD5 (-), CD22, CD23 (-), CD10 (-), CDllc, CD25, Cd103
Marginal Zone Lymphoma	DR, CD 19, CD20, CD22, CD5 (-), CD23 (-), CD10 (-), CDllc, CD25 (-), CD103 (-)

Table 5. Immunophenotype of Common B-Cell Chronic Lymphoproliferative Disorders

Abbreviations : DR ; HLADR, S ; surface, (+) ; positive, (-) ; negative, (- / +) ; spectrum from negative to weak positive

Abbreviations

AITL: angio-immunoblastic T cell lymphoma. ALCL: anaplastic large T cell lymphoma, ATLL: adult T cell leukaemic lymphoma, CLPD: chronic lymphoproliferative disorder. CTCL: cutaneous T cell lymphoma, EATCL: enteropathy associated T cell lymphoma, HS$\gamma\delta$TCL: hepato splenic gamma delta T cell lymphoma. LOLL: large granular lymphocytic leukemia, NHS$\gamma\delta$TCL: non-hepato-splenic gamma delta T cell lymphoma. PTCL: peripheral T cell lymphoma. SC – TCL: subcutaneous T cell lymphoma, T – PLL: T cell prolymphocytic lymphoma.

CONCLUSIONS

In acute leukaemias, immunophenotyping has a great role to play in defining as well as classifying this group entity as many a times. It is not possible to specify the blasts into myeloid or lymphoid lineage by evaluating their morphology or cytochemical staining characteristics. Similarly flow cytometric immunophenotyping has become an integral part of investigation of mature lymphoid neoplasms in clinical practice, precisely in the diagnosis, classification, staging and monitoring of treatment response. Needless to say that immunophenotyping has a crucial role in characterizing and distinguishing plasma cell neoplasms also. FCI allows detection of maturation asynchrony and aberrant antigen expression on multiple bone marrow lineages even in the absence of overt morphologic dysplasia and increased blast percentages thus helps in identifying and characterizing the myelodysplastic group of disorders, thus immunophenotypic analysis is of immense help in today's perspective for studying and evaluating all and every aspect of haematolymphoid malignancies.

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