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Immunophenotyping in Acute leukemias: First tertiary care centre experience from Punjab

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Abstract

Acute leukemia is a heterogenous group of disorders, require multidiscplinary approach for diagnosis and prognostication. Immmunophenotyping has emerged as important tool in diagnosis of hematolymphoid malignancies. Since, Punjab is emerging as cancer capital of India, with leukemia cases on the rise. The diagnostic modalities like flow cytometry need to be popularized and more medical fraternity is required to be familiar with this technique. Therefore we hereby present study from the state of Punjab focussing on leukemia patterns diagnosed by flow cytometry.

Keywords: Leukemia, Diagnosis, Flow Cytometry, Punjab.

Introduction

The state of Punjab is emerging as cancer capital of India, thus the medical fraternity needs to be made aware of the modalities available for cancer diagnosis. Apart from solid organ malignancies, the blood cancer (acute leukemia) incidence is also rising in Punjab. Acute leukemias are a heterogeneous group of malignancies presenting with different morphological, immunological and molecular characteristics. Though the gold standard methods like, microscopic examination of peripheral blood and bone marrow films may provide a provisional diagnosis of acute myeloid leukemia, acute lymphoblastic leukemia and lymphoproliferative disorders but remain unreliable if not supported by immunophenotyping. As per 2008 World Health Organization (WHO) classification of hematolymphoid malignancies immunophenotyping is mandatory for the diagnosis of each disease entity. Several advances in flow cytometry have improved the utility of flow cytometry in the diagnosis and classification of leukemia. The understanding of various phenotypic patterns of differentiation not only allows for more accurate diagnosis of leukemia but can also define complex antigenic profiles that are associated with specific molecular defects and thus playing an important role in deciding treatment strategies.[1,2] Despite the advantages of the flow cytometry and proven utility in leukemia diagnosis very few tertiary care centers in Punjab provide facility of leukemia diagnosis.

Materials and Method

A total of 100 patients of adult and paediatric acute leukemias, diagnosed in the Department of Pathology, Dayanand Medical College and Hospital, Ludhiana, during June 2014 to May 2015 were retrospectively analysed. In addition to routine evaluation by complete blood counts, the patients were evaluated with peripheral blood films, bone marrow aspirate & trephine biopsy, using May Grunwald Giemsa, Hematoxylin & Eosin and cytochemical stains (Myeloperoxidase and Periodic acid Schiff's). Bone marrow aspirate and/or peripheral blood samples collected from all the patients were processed, stained with 4 colors combination of antibody with standardized "stain-lyse-wash" technique and acquired on recalibrated 2 laser 6 color BD FACS Canto II flow cytometer. The calibration was done using 7 Color set up beads and Cytometer Setup and tracking beads.

Immunophenotyping was done in mononuclear cell obtained by lysing whole blood by BD FACS lysing solution. For immunophenotyping various combination of flurochrome (fluorescein isothiocyanate (FITC), phycoerythrin (PE), Allophycocyanine (APC) or peridinin chlorophyll protein (PerCP), conjugated monoclonal antibodies (MoAbs) were added per tube in sample. Data were acquired and blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC) and also a second gate was based on cell forward scatter angle, (FSC-A) versus SSC-A. The patients consent was taken before every bone marrow examination. This is a descriptive analysis and hence no statistics was involved.

Results

Over a period of one year, 100 patients were diagnosed as acute leukemia. Diagnosis was based on morphology, cytochemistry and immunophe-notyping by flow cytometer. There were 76males and 24 females with M:F = 3.1:1. Seventy five (75/100) were adults & 25% (25/100) were paediatric cases. Of the pediatric cases 14 (56%) were B-ALL, 8 (32%) T-ALL and 3 (12%) AML.

In 100cases of acute leukemia, 47were classified as acute myeloid leukemiaand 53were acute lymphoblastic leukemia (ALL). Immunophenotyping done in all the 47 cases for definite diagnosis and further subcategorisation showed side scatter low and CD45 dim events to be

consistently positive for CD 117, CD33 and CD13, CD34, HLADR and MPO. HLADR and CD34 highlighted the immaturity while CD117, CD13, CD33 and MPO were considered as myeloid lineage specific markers. CD33and CD117 were the myeloid markers that most commonly present in all AML subtypes, it's percent was 89.4%, 98.2%. CD13 was the next most commonly expressed antigen showing 88% positivity in all AML categories. CD14 and CD64positivity were more commonly associated with the monocyticleukemias (100% and 100%, respectively). (Fig. 1)

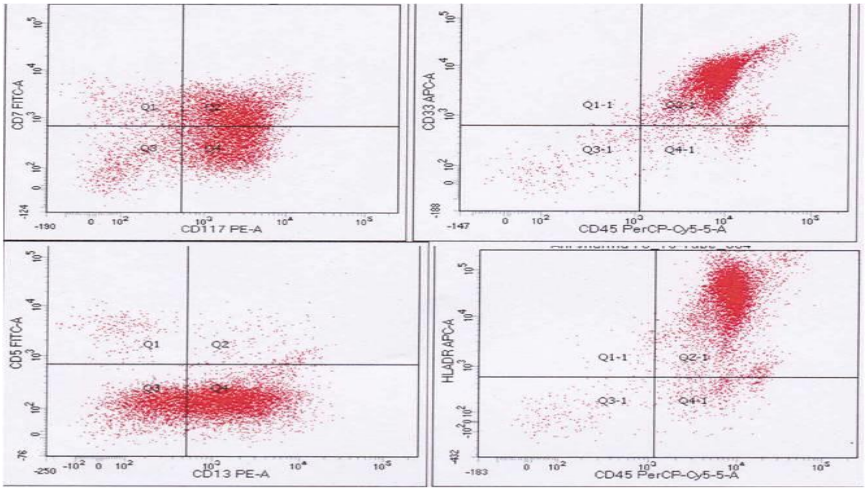

Fig. 1: Scattergram showing SSC A low and CD 45 dim events to be positive for CD13, CD33, HLADR and CD 117 along with aberrant CD7 expression in a case of acute myeloid leukemia

In addition to the 4 cases morphologically showing monocytic lineage cells another 8 cases on immunophenotyping showed monocytic lineage blasts positive for CD14, CD64 and CD11b. Hence of the total 47 cases, 12 cases were diagnosed as AML with monocytic differentiation.

Four cases morphologically suspected as Acute Promyelocytic Leukemia (APML) showed positivity for CD117, CD33, CD13 and MPO while were negative for HLADR and CD34 i.e. correlating the morphological diagnosis.

One case of AML M6 suspected morphologically showed Myeloid lineage blasts comprising 22% of the acquired events and remaining (78%) events were negative for CD45 and other leukemia specific markers. Since we did not do markers specific for erythroid lineage, diagnosis was made based on morphological correlation.

Of the total 53 cases, 44 cases were diagnosed as Precursor B cell lineage lymphoblastic leukemia as blast events showed positivity for CD 19 (100%) and CD 79a (100%). In addition CD10 (80%) positivity was also a consistent finding in majority of cases and HLADR, Tdt and CD34 positivity highlighted the immature phenotype of the blast events.(Fig. 2)

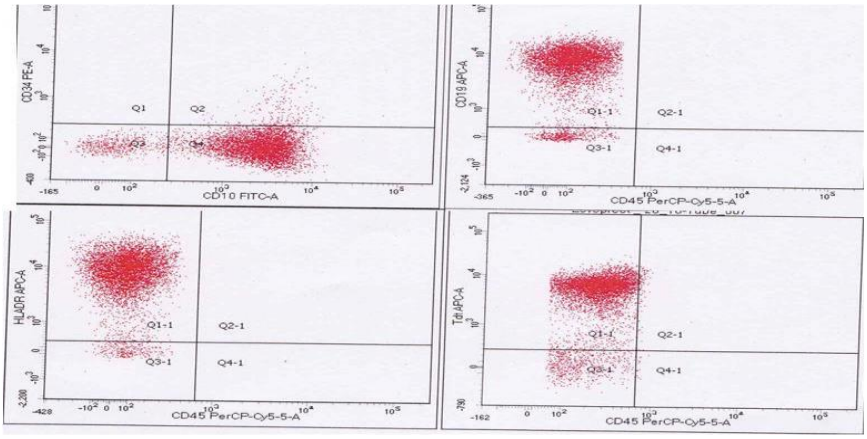

Fig. 2: Scattergram showing SSC A low and CD45 dim events to be positive for CD 10, CD19 and Tdtina case of Precursor B lymphoblastic leukemia

Nine cases showed blast events to be positive for CD5, CD 7 and cytoplasmic CD. Immaturity markers like CD34 (86%) and Tdt (80%) were also positive, hence were diagnosed as Precursor T lymphoblastic leukemia.

Of the total 100 cases, aberrant immunophenotypic expression was noted in 6 cases of AML (4 cases exhibited aberrant CD7 expression and 2 cases showed aberrant CD19 expression) and 10 cases of ALL (6 cases of Precursor B lymphoblastic leukemia showed aberrant CD33 expression while three showed aberreant CD13 expression and 1 case of Precursor T lymphoblastic leukemia showed aberrant CD 117 expression)

MPO staining was analysed in three categories with Category 1 forming majority of cases followed by Category 3 and Category 2 respectively.

Of the 20 cases in **Category 1** (Positive cytochemically and immunophenotypically) final impression of AML with maturation (AML M2) was given in 12 Cases while 4 cases were interpreted as AML with monocytic differentiation (AML M4/M5) and 4 cases as APML.

Of the 14 cases in **Category 3** (Negative both cytochemically and immunophenotypically), 9 cases were diagnosed as AML with minimal differentiation (AML M0), 4 cases were diagnosed as AML with monocytic differentiation(AML M4/M5) and one case was diagnosed as Acute erythroid leukemia (AML M6).

Of the 8 cases in **Category 2 (**Negative cytochemically and Positive immunophenotypically), 4 cases were diagnosed as AML with monocytic differentiation while remaining 4 cases were diagnosed as AML without maturation (AML M1).

The above observations show that MPO can be variable both cytochemically as well as immunophenotypically**.**

Discussion

Acute leukemias account for 352,000 new cases and 265,000 deaths worldwide in 2012.[2,3] The incidence is also on rise in North India with Punjab emerging as cancer capital of the country. Considering the poor prognosis early and accurate diagnosis of leukemia can reduce the morbidity and mortality rate in developing countries like India. The immunophenotyping has emerged as important tool in diagnosing acute leukemia. The present study results demonstrated that the percentage of ALL among children was 72% whereas the percentage of AML in adults was 79.5%. Our study showed that 79.3% of ALL cases as B-cell lineage whereas 20.7% as T- ALL. This is supported by a study from BP Koirala Memorial Cancer Hospital in Nepal during 2- year period (2010 – 2012), which reported that out of total 52 new cases of acute leukemia, 64.5% were B-ALL and 35.5% labelled as T-ALL.(5) B-ALL is diagnosed if B-cell markers (CD19, cytoplasmic CD79a, cytoplasmicCD22) are expressed in combination and the diagnosis of ALL of T-cell lineage is based on the presence of cytoplasmic CD3 in all blasts with coexpression of CD5. In the present study, CD19, CD22 and cytoplasmic CD79a were expressed in virtually all cases of B-cell ALL. Andcytoplasmic CD3 and CD5 were seen in almost all cases of T-ALL.

Salem et al. showed that cytoplasmic CD79a and CD19 were the most sensitive marker for B-ALL while cytoplasmic CD3 and CD5 were the most sensitive markers for T-ALL.[3,4]

Peffault de Latour RPD et al observed that flow cytometry is more sensitive and superior than cytochemistry and can be more beneficial if the cutoff for a MPO positive value is lowered to 3% of blast population rather than 10%.[4]

Flow cytometry in our study showed CD45 consistently positive in the all, CD117, CD33 and CD13, CD34, HLADR and MPO. HLADR and CD34 highlighted the immaturity while CD117, CD13, CD33 and MPO were considered as myeloidlineage specific markers.

A study by Legrand O et al also showed similar results showing consistent positivity for myeloid lineage antigens CD13 (95%), CD33 (91%), and MPO (73%) and the hematopoietic progenitor cell markers HLA-DR (87%), CD117 (73%), and CD34 (68%).[5] Salem D A et al also had concordant results with our study who observed CD33 as the most commonly myeloid marker (89.4%) present in all AML subtypes.

During the present study CD13 was expressed in 77.9% cases in all AML categories, while expression of CD117 was seen in 74.3% of AML. CD14 and 64 positivity were more commonly associated with the monocyticleukemias (64.1 and 61.5%, respectively). Of 39 cases of AML, Acute promyelocytic leukemia was diagnosed in only 3 cases (7.6%).[6]

Poeta et al. observed that CD7 positivity was significantly associated with leucocytosis and also overall survival and disease free survival rate of CD7positive AML was lower than those of CD7 negative patients. However no correlation was found between CD7 expression with age, sex, hepatomaegaly and central nervous system involvement.[7] Kita K et al also found that CD7 expression on AML cells is indicative not only of phenotypic, but also of functional immaturity and can be regarded as a prognostic risk factor in AML.[8] In contrast study done by Ball et al concluded that patients which were positive for CD2 and CD19 had actually higher complete remission rate and overall survival.[9]

Study done by Khan AH et al. on Acute Lypmphoblastic Leukemia of northern India also showed two fold male predominance, with 27 females and 48 males of total 75 cases. The age group was ranging from a minimum of 2 to a maximum of 64 years (mean age 27.9 years).[10]

Snower DPin which sensitivity and specificity of PAS alone for lymphoblastic leukemia was 52% (15 true positive out of 29) and 81% (4 false positive)

respectively and also the study concluded that PAS positive alone with other negative stains (MPO, SBB) continues to have a role in distinction between lymphoblastic and myeloid leukemia.[11]

Khan AH et al reported 72%(29) cases with B cell phenotype while 28%(11) case had T cell phenotype. B-ALL was predominantly seen in children while T cell ALL was predominantly seen in adults.[10]

Shrestha S et al also highlighted that if CD22 or CD79a expression is found either cytoplasmic or on the cell surface with the expression of CD19 and HLA-DR and also B-ALL with CD10 positivity has better prognosis than B-ALL with CD10 negative.[12] In our study, 24 cases of B-ALL were found to be positive for Tdt. Michiels JJ in his study observed that cytogenetic analysis done in such cases revealed underlying trisomy 12 and monosomy of the long arm of chromosome 6.[13]

The present study also showed six cases of acute lymphoblastic leukemia with aberrant CD33 expression and one case with aberrant CD117 expression. These markers indicate a poor prognosis and a poor response to drug therapies targeting conventional ALL according to study done by Suggs JL et al.[14]

Conclusion

Flow cytometry has emerged as an integral part in leukemia diagnosis. It is essential in lineage assessment and definite diagnosis of leukemia. Punjab is emerging as a cancer capital of India and thus the diagnostic facilities for the cancer should include flow cytometry for early detection of leukemias.

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