Immunophenotypic Aberrancies in Acute Leukemia: A Tertiary Care Centre Experience

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ABSTRACT

Introduction: Acute leukaemia’s (AL) are a heterogeneous group of haematological malignancies with presence of 20% or more blasts in peripheral blood, bone marrow. Malignant cells display characteristic patterns of surface antigenic expression. Aberrant phenotypes in are defined as patterns of antigen expression on neoplastic cells different from the process of normal hematopoietic maturation.

Aim: To evaluate the occurrence of aberrant phenotype in newly diagnosed cases of AL and

Material and Methods: The study included 100 patients in whom both bone marrow aspiration and flowcytometry were performed. patients with blasts >20% of all age groups were included in the study. Flowcytometric analysis (FCA) was done using the monoclonal antibody panel of on Peripheral blood/ Bone marrow.

Observation: Out of 100 cases of, 53 cases were categorised as AML, 43 cases as ALL and 4 cases MPAL. ALL were subcategorised on the basis of IPT into B-ALL and T-ALL which comprised of 88 % and 12% total ALL (43%) cases.

Discussion: CD33 and CD13 were the most commonly expressed antigens in AML with CD7 being the most common aberrancy. CD19 was expressed in all cases of B-ALL followed by cCD79a , CD10 , Tdt (86.8%) with CD13 being the most common aberrancy in B-ALL . cCD3, CD7 and CD5 were expressed in all cases of T-ALL with aberrant antigen expression in 80% of cases of T-ALL. MPAL cases showed expression of B / Myeloid antigens.

Conclusion: The diagnosis and classification of leukaemia rely on simultaneous application of cytomorphology, cytochemistry, FCM, cytogenetics and molecular techniques. Flowcytometry is of great help in the diagnosis of AL particularly in ALL for lineage
INTRODUCTION

They present with varying clinical, morphologic, immunologic and molecular features. Malignant cells display characteristic patterns of surface antigenic expression. Immunophenotyping of leukaemia cell results in broad classification of acute leukemia into Acute Myeloid Leukaemia (AML), Acute Lymphoid Leukaemia (ALL) and Mixed Phenotypic Acute Leukaemia (MPAL) based on the expression of different subsets of surface molecules now defined as Cluster of differentiation (CD) Antigens. Flowcytometry is used to study the antigenic expression of CD markers on leukocytes.\(^1\)

Aberrant phenotypes in AL are defined as patterns of antigen expression on neoplastic cells different from the process of normal hematopoietic maturation. Aberrant expressions of antigen in AL include lineage infidelity (lymphoid marker expression in myeloid blast cells like expression of CD7, CD19, CD79a, CD10, CD2, CD5, CD3), asynchronous antigen expression (coexistence of early and late markers in one cell like CD34 and CD15 in AML), antigen over expression; which is abnormally increased expression of certain antigen per cell, aberrant light scatter properties; which involves the expression of lymphoid associated antigens in blast cells displaying relatively high forward scatter (FSC) and side scatter (SSC) corresponding to normal myeloid cells and absence of lineage specific antigens; which involves absence of expected antigen expression (CD13 and CD33 on myeloid blasts). In
ALL these aberrancies include cross-lineage antigen expression (expression of myeloid antigens in ALL, B-lineage antigens in T-ALL or T-lineage antigens in B-ALL).²

Aberrant phenotypes can be used for minimal residual disease (MRD) detection and monitoring. Minimal residual disease is defined as persistence of leukemic cells after chemotherapy that cannot be identified with routine morphologic evaluation.³

With various modifications in flowcytometric instrument and availability of wide range of antibodies and fluorochromes have changed our perception in identification of normal cell population and aberrancies present on leukemic cells. The present study is done to evaluate the occurrence of aberrant phenotype in newly diagnosed cases of AL.

MATERIAL AND METHODS

This prospective study conducted in the Department of Pathology of a tertiary care centre in North India and included 100 newly diagnosed cases of AL during the period of June 2017 to Dec 2018. Diagnosis of AL was done based on morphological examination of Leishman stained peripheral blood smears and bone marrow aspiration smears, cytochemistry (SBB, PAS) and flowcytometric immunophenotyping.

Flowcytometric analysis (FCA) was done using the monoclonal antibody panel of AL on Peripheral blood/ Bone marrow using a standard stain-lyse-wash method. Listmode data was acquired on FACS Canto II Flowcytometer (Becton Dickinson, San Jose, CA) and analysed by FACS Diva software. Results were obtained by gating the blasts cells with side scatter (SSC) versus forward scatter (FSC) followed by SSC versus CD45 gating. For surface antigen, marker positivity was considered when >20 % blasts cells were positive and > 10 % blast cell positivity was considered for cytoplasmic antigen.

All the quality control measures were undertaken before starting the procedure such as checking the system pressure and vacuum gauges, checking the optical alignment of red and blue laser, titration of antibodies for fluorescence standardization, colour compensation using 7 colour set up beads of instrument and verification of antibody integrity using quality control protocol.⁴

Required approval from institutional ethical committee of University of Health Sciences was taken. Informed consents were obtained from all participants after explanation of the study.
After haematological study and flow cytometry, the biomedical waste generated during the procedure were discarded as per biomedical waste (management and handling) rules, 2016.5

**Statistical analysis**

SPSS statistical software 20.0 was used for statistical analysis. Quantitative (Continuous) variables were expressed as mean. Categorical variables were expressed as frequencies and percentage and nominal categorical data between the groups were compared using chi-squared test. In all statistical tests, P value <0.5 was considered significant. Association of aberrant antigen expression in cases was correlated with clinical, morphological and cytochemistry findings by using Chi Square test.

**RESULTS**

Out of total of 100 patients of AL, 53 cases were categorised as AML, 43 cases as ALL and 4 cases as MPAL. ALL was subtyped into B-ALL and T-ALL with 38 cases (88%) and 5 cases (12%) in each respectively. Fever (76%) was the most common presenting complaint followed by bleeding manifestations (25%) and gum hypertrophy (10%). On examination pallor was observed in 90% cases, organomegaly (Hepatosplenomegaly) in 65% and lymphadenopathy in 21% cases. Clinical features in AL are shown in Table 1.

**Table 1 : Clinical Features of Acute Leukemia cases**

| Clinical Features       | AML (N=53) | ALL (N=43) | MPAL (N=4) | Total (N=100) |
|-------------------------|------------|------------|------------|---------------|
| Pallor                  | 49 (92.4%) | 37 (86%)   | 4 (100%)   | 90 (90%)      |
| Fever                   | 35 (66%)   | 38 (88.4%) | 3 (75%)    | 76 (76%)      |
| Organomegaly (Hepatosplenomegaly) | 38 (71.7%) | 24 (55.8%) | 3 (75%)    | 65 (65%)      |
| Bleeding                | 11 (20.7%) | 12 (27.9%) | 2 (50%)    | 25 (25%)      |
| Lymphadenopathy         | 2 (3.7%)   | 16 (37.2%) | 3 (75%)    | 21 (21%)      |
| Rash                    | 4 (7.5%)   | 5 (11.6%)  | 2 (50%)    | 11 (11%)      |
| Gum Hypertrophy         | 5 (9.4%)   | 4 (9.3%)   | 1 (25%)    | 10 (10%)      |
Cytochemical staining with Myeloperoxidase (MPO) and Sudan black B (SBB) coupled together was positive in 41 (77.3%) cases of AML. PAS (Periodic Acid Schiff) staining was positive in 36 (83.7%) cases of ALL. MPO and SBB was positive in 1 (25%) case and PAS was positive in 1 (25%) case of MPAL. Two (50%) cases of MPAL showed positive expression of MPO, SBB and PAS.

CD45, an immunological marker was used for gating purpose for the identification of blast cells by FCM. In AML (53%), CD33 and CD13 were the most commonly expressed (98%) antigens followed by MPO (88.6%), CD117 (73.5%), CD64 (64%) and CD34 (64%). In B-ALL (38%), CD19 was expressed in all (100%) cases followed by cCD79a (92%), CD10 (94.7%), Tdt (86.8%), CD20 (84%) and CD34 (65.7%). In T-ALL (5%), cCD3, CD7 and CD5 were expressed in all (100%) cases followed by CD4 (80%) and CD8 (20%). In MPAL (4%), all were B/Myeloid with 100% expression of CD13, CD33, MPO, CD19, CD79a, CD34 and HLA-DR.

In AML, 14 (27%) out of 53 cases showed aberrant lymphoid antigen expression. CD7 was the most common aberrant antigen expressed in 9 (17%) cases followed by CD19 in 3 (6%) cases and CD10 in 2 (4%) cases. Co expression of HLA-DR and CD34 was seen in about two thirds of patients (64%) with AML in our study. In B-ALL, 21 (55%) out of 38 cases expressed aberrant antigens with CD13 (50%) being the most common expressed aberrant antigen followed by CD33 (3%), CD7 (3%) and CD5 (3%). Asynchronous dual expression of CD34 and CD20 was found in 20 (53%) cases of B-ALL. In T-ALL, 4 (80%) out of 5 cases expressed aberrant antigens with CD117, CD19, CD20 and CD10 being expressed in one case each of T-ALL. Aberrant expression of CD markers in AML, B-ALL and T-ALL are shown in Table 2,3 and 4.

Table 2 : Aberrant Antigen Expression in AML cases (n=14)

| ABERRANT ANTIGEN | No of Cases/ total aberrant cases | Percentage ( n=53) |
|------------------|----------------------------------|--------------------|
| T + Ly AML       |                                  |                    |
| CD 7             | 9/14                             | 17%                |
| B+ Ly AML        |                                  |                    |
| CD 19            | 3/14                             | 6%                 |
| CD 10            | 2/14                             | 4%                 |
TABLE 3: Aberrant Antigen expression in B-ALL cases (n=38)

| ABERRANT ANTIGEN | NO of CASES | PERCENTAGE |
|------------------|-------------|------------|
| CD34,CD20        | 20          | 53%        |
| CD 13            | 19          | 50 %       |
| CD 117           | 2           | 6%         |
| CD 33            | 1           | 3%         |
| CD13,CD33        | 1           | 3%         |
| CD 13,CD5        | 1           | 3%         |
| CD117,C7         | 1           | 3%         |
| CD 7             | 1           | 3%         |
| CD 5             | 1           | 3%         |

TABLE 4: Aberrant Antigen expression in T-ALL cases (n=5)

| ABERRANT ANTIGEN | NO of CASES | Positive Rate |
|------------------|-------------|---------------|
| CD 117           | 1           | 20%           |
| CD 19            | 1           | 20%           |
| CD 20            | 1           | 20%           |
| CD 10            | 1           | 20%           |

DISCUSSION

In India, the incidence of ALL and AML are 35 % and 15% of all haematological malignancies respectively.6
We performed this study to categorize by cytomorphology into lymphoid and myeloid, correlate immunophenotyping profile of with cytomorphological category and evaluate frequency of aberrant phenotype by flowcytometry.

There is variable expression of lymphoid antigen in AML based on range of markers studied, total number of samples and criteria of aberrancy used. In our study, 53 patients were diagnosed as AML, out of which 14 (27%) patients showed aberrant antigen expression. CD7 was the most common aberrant antigen expressed in 9 (17 %) cases followed by CD19 in 3 (6 %) cases and CD10 in 2 (4 %) cases.

Also reported CD7 as the most common aberrant lymphoid antigen. T cells, NK cells and stem cells expressed CD7 which is an activation and adhesion molecule. CD7 expression likely to represent an origin form an early stem cell in myeloid development and is associated with immaturity markers CD34 and HLA-DR, antigen receptor gene rearrangements and lower chances of complete remission and overall poor prognosis.7

There is no consensus in clinical relevance of lymphoid antigen expression in AML. Some studies7,9 reported Ly+AML to be associated with poor prognosis but some reported it to be associated with favourable prognosis whereas other suggest it to be of no prognostic value.10

The low frequency of aberrant antigen expression in AML in our study as compared to published literature may be related to technical issues (differences in the number of patients tested for those markers in this study compared to other studies) or difference in laboratory protocols or due to ethnical variation.

Degree of myeloid antigen expression in ALL cases varied from 4.3%-64%. The reasons for this variation may be due to clones of monoclonal antibodies used having different binding characteristics, variation in threshold for antigen positivity and different flowcytometry methods used in sample processing, make of instruments and reagents.

In our study, 21(55%) cases of total B-ALL cases expressed aberrant antigens with CD13 (50 %) being the most common expressed aberrant antigen followed by CD33 (3%), CD7 (3%) and CD5 (3%). Our findings were in consistent with Sharma et al12 (42.5%). Salem et al13 reported slightly lower incidence of 10.5% while Seegmiller et al2 reported a high incidence of 86.5% aberrant antigen expression in B-ALL.

Most of the studies4, 2, 12, 13,14, reported CD13 and CD33 as the most common aberrant myeloid antigen in ALL which was also observed in our study. However, the clinical significance of expression of myeloid antigen in B-ALL is variable in literature. Belurkar et
al and Seegmiller et al did not find any significant effect of aberrant myeloid antigen expression on clinical presentation, relapse rate and survival except their role in minimal residual disease detection in B-ALL.

Sharma et al found that adult My+ALL group was associated with statistically significant lower WBC count and lower peripheral blast count while in paediatric cases it is associated with lymphadenopathy, lower blast count and higher expression of CD34 while Rodriguez et al found a statistically significant association between presence of myeloid markers in adult ALL with inferior disease free survival and shorter survival. In contrast to these studies, Lopes et al found that platelet count was significantly lower in group without aberrant myeloid expression and may indicate a greater risk of bleeding during treatment in this group. In our study, B-ALL patients with aberrant antigen expression showed association with poor prognostic factors although did not achieved statistical significance.

In T-ALL, 4 (80%) of our cases expressed aberrant antigens with CD 117, CD 19, CD20 and CD10 being expressed in one case each of T-ALL which was higher than the published literature. Higher incidence of aberrancy in present study could be due to small number of cases in this group.

Limitation of our study is, we were not able to correlate with treatment response parameters to definitely comment upon overall prognostic significance of expression of aberrant markers in AL. However; evaluation of the expression of IPT aberrancies remain valuable for more precise characterization of leukemic population and can be helpful for making alternate therapeutic decisions and monitoring of minimal residual disease during the course of the disease.

**CONCLUSION**

Simultaneous application of multiple techniques are used now a days for the diagnosis and classification of leukaemia. Correlation of cytomorphology, cytochemistry, FCM, cytogenetics and molecular techniques are required to assign the diagnostic sample to the correct entity.

FCM is of great help in the diagnosis of AL particularly in ALL for lineage assignment and in classifying the unclassifiable cases based on morphology and cytochemistry like, the MPA and also helps in detecting aberrant antigen expression in acute leukemia. Evaluation of the expression of IPT aberrancies remain valuable for more precise characterization of leukemic
population can be helpful for making alternate therapeutic decisions and monitoring of minimal residual disease during the course of the disease.

Our results point to the need for a more extensive and elaborated study to evaluate the potential prognostic significance of aberrant marker expression in AL. The presence of aberrancies in strongly supports the continued use of FCM in the diagnosis and monitoring of AL.

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Fig 1: AML with aberrant expression of CD7
**Figure 1:** AML with aberrant expression of CD7, Blasts are positive for CD13, CD33, CD34, CD117, CD64, HLA-DR, CD7, Dim positive for CD45, negative for CD8

**Fig 2:** B-ALL with aberrant expression of CD13

**Figure 2:** B-ALL with aberrant expression of CD13, Blasts are positive for CD19, CD10, cCD79a, HLA-DR, CD13, Negative to Dim positive for CD45, negative for cMPO, CD64, CD33, cCD3
Figure 3: T-ALL with aberrant expression of CD19, Blasts are positive for CD7, CD34, CD8, CD64, cCD3, CD19, Dim positive for CD45, negative for CD64, CD10, cMPO