

Original Research Article

Characterization of Immunophenotyping in ALL: A Single Center Study of Western India

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Abstract: This retrospective observational study was done among 227 ALL cases at a cancer treatment center in Western India. Data were collected retrospectively from hospital records during June 2015 to May 2016. Diagnosis of ALL was made based on the complete blood cell counts, peripheral blood smear/bone marrow aspirate smear and immunophenotyping study. Out of 227 ALL patients, 217 cases were of B-ALL, 8 cases were of T-ALL and 2 cases were of biphenotypic ALL. Of these 217 cases of B-ALL 190 (87.56) were pediatric age (range 0.5-15 years) and 27 (12.44) were adult (range 16-52 years). T-ALL cases were equally distributed in both age groups, 4 (50%) cases in pediatric age group (range 1-7 years) and 4 (50%) cases were adult (range 19-62 years). biphenotypic leukemia found in only children (2 (100%) case, one was 3 year old male and another was 5 years age male). The frequency of B-cell marker in B-ALL was found to be 217 (100%) for CD 19, 193 (88.94%) for CD 10, 162 (74.65%) for CD 79a and 35 (16.13%) for CD 20. CD 34 expression was found in 37 (17.05%) cases of B-ALL. HLA-DR was found in 30 (13.82%) cases while TdT was found in 27 (12.44%) cases. Aberrant expression of myeloid antigens was found in 57 (26.27%) cases of B-ALL. Among T-ALL, the positivity of CD 3 and CD 7 was 100 % (8/8 cases) while CD 5 was positive in 6 (75%) cases. CD 34 expression was found in only one case of T-ALL. Two cases of ALL had biphenotypic leukemia with positive CD3, CD 7, CD 19, CD 20, HLA-DR and TdT. There was aberrant expression of myeloid antigen in B-ALL. CD 13 was found in 37 (17.05%) B-ALL and CD 33 was found in 20 (9.22%) B-ALL cases. None of the T-ALL case had aberrantly expressed myeloid marker. We concluded that immunophenotyping plays a key role in diagnosis of ALL.

Keywords: ALL, Immunophenotyping study, B-ALL, T-ALL, Biphenotyping leukemia**INTRODUCTION**

Acute lymphoblastic leukemia (ALL) is a malignancy of lymphoid precursor cells characterized by abnormal proliferation and accumulation of immature lymphoid precursor cells at various differentiation stages [1]. ALL is the most common form of childhood malignancy and these days it has become a curable disorder in most of the cases. In children B-ALL are most common (80-85%) and T-ALL consists of about 15% of ALL cases [2, 3]. The treatment and prognosis of ALL depends on its type (B-ALL or T-ALL).

The older classification of ALL, French American British (FAB) classification, [4] was based on morphology however immunophenotyping helps in

defining ALL by the antigenic expression of malignant cells. Flowcytometry (FCM) is a newer technique to identify and quantify such antigens (Immunophenotyping) [5]. This immunophenotyping studies give useful information about heterogeneity of ALL and also helps in diagnosis, treatment and prognostic evaluation of ALL [5].

Various studies have shown the importance of flowcytometry/immunophenotyping of ALL in determining treatment and to ascertain prognosis [5-14].

In view of scarcity of data available regarding immunophenotypic profile of ALL patients from Western India, this study was an endeavor to evaluate

immunophenotypic profile of ALL patients at our cancer treatment center.

MATERIAL AND METHODS

This retrospective observational study was done among 227 ALL cases at a cancer treatment center in Western India. Data were collected retrospectively from hospital records during June 2015 to May 2016 after due permission of competent authorities.

At our center, the diagnosis of acute leukemia was made based on the complete blood cell counts, peripheral blood smear/bone marrow aspirate smear and immunophenotyping study. Blood smear/ bone marrow smear analysis was performed by pathologist who was blinded to the clinical state of the patient and acute leukemia was diagnosed only if blast count was >20%.

Total 227 patient samples (200 from peripheral blood and 27 from bone marrow aspirate) were collected in ethylenediaminetetraacetic acid (EDTA) and processed for flowcytometry within 3 hours of collection, at our institutional lab, by a pathologist who was blinded to the clinical state of the patient. Diagnosis of ALL was established on the basis of morphological and immunophenotypic criterias [15]. The ALL was classified in to B-ALL or T-ALL based on WHO criteria [15] Biphenotypic leukemia was diagnosed as per EGIL criteria [16].

For the immunophenotypic diagnosis of acute leukemias, a combination of four fluorochrome-conjugated monoclonal antibodies (MoAb) per tube was added to the samples. All the MoAbs were obtained from Becton Dickinson (San José, California, United States). They were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) or allophycocyanin (APC), and were directed to antigens for T cells (CD3, CD5, CD7, cytoplasmic (c) CD3), B cells (CD10, CD19, CD20, cCD79a), myeloid cells [CD13, CD33, CD117 and myeloperoxidase (MPO)], monocytes (CD14), erythroid cells (alpha-glycophorin), platelet cells (CD61), non-specific lineage pan-leukocytes (CD45) and precursor cells [CD34, human leukocyte antigen-DR (HLA-DR) and terminal deoxynucleotidyl transferase (TdT)].

Membrane and intracytoplasmic labeling was performed using 1×10^6 cells per tube. For membrane labeling, the samples were incubated with each antibody for 20 minutes. The erythrocytes were lysed with 2 ml of FACS lysing solution (Becton Dickinson, San José, California, United States), diluted to 1:10 and then washed with 2 ml of phosphate-buffered saline (PBS; pH = 7.4). For intracytoplasmic labeling, FACS permeabilizing solution (Becton Dickinson, San José)

was used in accordance with the manufacturer's instructions.

Data acquisition and sample analysis was performed in a FACSCalibur flow cytometer (Becton Dickinson, San José), using the CellQuest software (Becton Dickinson, San José), after calibration with the Calibrite bead kit (Becton Dickinson, San José), using the FACSComp program (Becton Dickinson, San José).

Lymphocyte labeling with CD4 FITC/CD8 PE/CD3 PerCP was used, to compensate for fluorescence and eliminate overlapping before data acquisition. The blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC) and also a second gate considering cell size (forward scatter angle, FSC) versus SSC. A total of 20,000 events were acquired in the target gate. Negative controls using isotype IgG1 and IgG2a monoclonal antibodies were run in all cases. The criteria used for determining antigen positivity included analysis of negative controls and expression of the marker by more than 20% of the gated cells. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed the particular aberrant marker. In ALL, aberrant expression of CD33 and CD13 were analyzed.

Biphenotypic acute leukemia (BAL) was diagnosed as per EGIL scoring system [16]. According to this scoring system, a case was considered as biphenotypic when point values were greater than two for myeloid and one for the lymphoid lineages [16].

RESULTS

Total 227 ALL patients were included in this study. Out of them 217 cases were of B-ALL, 8 cases were of T-ALL and 2 cases were of biphenotypic ALL.

Of these 217 cases of B-ALL 190 (87.56) were pediatric age (range 0.5-15 years) and 27 (12.44) were adult (range 16-52 years). T-ALL cases were equally distributed in both age groups, 4 (50%) cases in pediatric age group (range 1-7 years) and 4 (50%) cases were adult (range 19-62 years). biphenotypic leukemia found in only children (2 (100%) case, one was 3 year old male and another was 5 years age male). (Table No.1)

Immunophenotype Markers (Table 2)

The frequency of B-cell marker in B-ALL was found to be 217 (100%) for CD 19, 193 (88.94%) for CD 10, 162 (74.65%) for CD 79a and 35 (16.13%) for CD 20. CD 34 expression was found in 37 (17.05%) cases of B-ALL. HLA-DR was found in 30 (13.82%) cases while TdT was found in 27 (12.44%) cases. Aberrant expression of myeloid antigens was found in 57 (26.27%) cases of B-ALL.

Among T-ALL, the positivity of CD 3 and CD 7 was 100 % (8/8 cases) while CD 5 was positive in 6 (75%) cases. CD 34 expression was found in only one case of T-ALL. Two cases of ALL had biphenotypic leukemia with positive CD3, CD 7, CD 19, CD 20, HLA-DR and TdT.

Aberrant Expression Of Myeloid Antigen (Table 3, 4)

There was aberrant expression of myeloid antigen in B-ALL. CD 13 was found in 37 (17.05%) B-ALL and CD 33 was found in 20 (9.22%) B-ALL cases. None of the T-ALL case had aberrantly expressed myeloid marker.

Table 1: Age wise distribution of ALL cases (227)

| | B-cell (n 217,100%) | | T-cell (n 8,100%) | | Biphenotypic (n 2, 100%) | |
|-----------|---------------------|------------|-------------------|----------|--------------------------|------|
| | M | F | M | F | M | F |
| <15year | 141(08.21%) | 49(1.37%) | 3(12.5%) | 1(%) | 2(%) | 0(%) |
| >15 years | 19(5.93%) | 8(2.74%) | 3(37.5%) | 1(12.5%) | 0(%) | 0(%) |
| Total | 162(73.97%) | 57(26.03%) | 6(75%) | 2(25%) | 2 (100%) | 0(%) |

Table 2 : Immunophenotypic characteristics of ALL cases

| B-ALL (n=217) | |
|-----------------------|--------------|
| CD 10 | 193 (88.94%) |
| CD 19 | 217 (100%) |
| CD 20 | 35 (16.13%) |
| CD 79 | 162 (74.65%) |
| CD 34 | 37 (17.05%) |
| HLA-DR | 30 (13.82%) |
| TdT | 27 (12.44%) |
| Aberrant (13, 33) | 57 (26.27%) |
| CD 45 | 48 (22.12%) |
| CD 38 | 10 (4.60%) |
| T-ALL (n=8) | |
| CD 3 | 8 (100%) |
| CD5 | 6 (100%) |
| CD7 | 8 (100%) |
| CD 19 | 1(12.5%) |
| CD 34 | 1(12.5%) |
| HLA-DR | 2(25.0%) |
| TdT | 2(25.0%) |
| Biphenotype ALL (n=2) | |
| CD 3 | 2 (100%) |
| CD 7 | 2 (100%) |
| CD 19 | 2 (100%) |
| CD 20 | 2 (100%) |
| CD 79 | 2 (100%) |
| HLA-DR | 2 (100%) |
| TdT | 2 (100%) |
| MPO | 2 (100%) |

Table 3: Myelod-monocyticantigen expression in ALL

| | B-ALL (117, 100%) | T-ALL |
|--|-------------------|-------|
| | | |

| | | |
|-------|-------------|--------|
| MPO | 2 (0.017%) | 00(0%) |
| CD 13 | 37 (17.05%) | 00(0%) |
| CD 33 | 20 (9.22%) | 00(0%) |
| | | |

Table 4: Myeloid antigen expression in ALL

| | B-ALL (117, 100%) | T-ALL |
|---------------------|-------------------|--------|
| One antigen (CD13) | 37 (17.05%) | 00(0%) |
| One antigen (CD 33) | 20 (9.22%) | 00(0%) |

DISCUSSION

The present study was first of its kind from Rajasthan describing immunophenotypic profile of ALL cases. Most of the ALL cases were children (196/227, 86.34%) indicating higher frequency in children as observed in previous studies [17, 18]. Also the frequency of ALL was more in male compared to females in each age group which is also similar with previous studies [17, 19-20].

In current study, B-ALL was found in 95.59 % cases of ALL and T-ALL was found in 3.52 % cases, indicating B-ALL commoner than T-ALL. This finding also matches with previous reports [2, 3].

In B-ALL, CD 19 was found in all 217 cases. CD20 was found in only 35 (16.13%) cases. CD19 is used as the primary marker for identification of B-ALL as CD19 is the earliest B-lineage-specific antigen and it precedes the appearance of HLA-DR and B-specific antigens i.e. CD10, CD20. Because CD19 expression is maintained during B-cell neoplastic transformation, CD19 expression is useful in the diagnosis of B-ALL [21, 22]. A Chinese study also showed significantly higher positive rate of CD19 (115/116, 99.1%) than CD20 (33/116, 28.4%) in 116 cases with B-ALL ($P < 0.01$) [23].

In T-ALL CD3 was found in all cases. CD 3 is normally expressed on T-cells and is diagnostic of T-ALL [22, 24]. In our study CD 7 was also positive in all cases of T-ALL(100%). This is in accordance with previous studies which also detected CD 7 in T-ALL with frequencies >95% to 100% [25-27]. However CD7 positive blasts does not signify a diagnosis of T-ALL because CD 7 expression is also found in B-ALL and myeloid leukemias [28-29].

In this study, aberrant expression of myeloid antigen was also observed in 57 (26.27 %) B-ALL patients. CD 13 was the most common (37 case) followed by CD 33 (20 case). In previous studies, aberrant expression of CD 13 was found in 25-55% B-ALL and CD 33 was found in 23-45 % B-ALL cases [30-31]. In contrast to our study Bradstock *et al* identified CD33 as the most common form of aberrant antigen expression (11%), followed by CD13 (5%) [32-33].

We did not find CD 117 (a myeloid marker) expression in ALL cases. The expression of CD117 in ALL is a rare finding and its expression is associated with activating mutations of FLT3 [34]. Bhushan *et al.* and Suggs *et al* also did not find CD 117 in ALL cases [35-36].

Recently a report from North India had shown aberrant CD 117 positivity in their ALL patients [37]. A possible explanation of this deviation from our finding might be different geographic population.

CONCLUSION

Immunophenotyping plays a key role in diagnosis of ALL. In our study most of the ALL cases were children (196/227, 86.34%), B-ALL was found in 217/95.59 % cases.

The frequency of B-cell marker in B-ALL was 217 (100%) for CD 19, 193 (88.94%) for CD 10, 162 (74.65%) for CD 79 and 35 (16.13%) for CD 20. CD 34 expression was found in 37 (17.05%) cases of B-ALL. Aberrant expression of myeloid antigens, CD 13 was found in 23 (10.61%) B-ALL and CD 33 was found in 6 (2.76%) B-ALL cases while both of these (CD13 and CD 33) were co expressed in 14 (6.45%) B-ALL cases.

Among T-ALL, the positivity of CD 3 and CD 7 was 100 % (8/8 cases) while CD 5 was positive in 6 (75%) cases. CD 34 expression was found in only one case of T-ALL.

Two cases of ALL had biphenotypic leukemia with positive CD3, CD 7, CD 19 and CD 20.

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