

## Original Article

## Immunophenotypic analysis of B-cell acute lymphoblastic leukemia at a tertiary care hospital in Sri Lanka

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## ABSTRACT

**Objectives:** B-cell acute lymphoblastic leukemia (B-ALL) is a hematological malignancy that predominantly affects children and is characterized by the rapid proliferation of immature lymphoblasts. This study aims to describe the immunophenotypic characteristics of B-ALL in a cohort of patients diagnosed in a tertiary care hospital in Sri Lanka.

**Materials and Methods:** A descriptive analytical study was conducted on 47 B-ALL cases diagnosed between June 2021 and December 2024. Diagnosis was established through morphological examination and immunophenotypic analysis using multi parameter flow cytometry.

**Results:** The cohort's mean age was 35.1 years, with a near-equal gender distribution (51.1% female, 48.9% male). Immunophenotypic analysis by flow cytometry revealed cluster of differentiation (CD19) positivity in 97.9% of cases, CD10 in 89.4%, and CD34 in 97.9%. Aberrant myeloid markers CD13 and CD33 were present in 32% and 30% of cases, respectively. The majority (53.2%) were classified as typical B-ALL without aberrant markers.

**Conclusion:** The study highlights significant immunophenotypic heterogeneity in B-ALL among Sri Lankan patients, with a notable adult representation. The high expression of core B-cell antigens reinforces their diagnostic utility, while the presence of aberrant myeloid markers suggests underlying genetic complexities associated with poor prognostic factors. Further research is warranted to explore genetic profiling and its implications for treatment outcomes.

**Keywords:** Aberrant expression of cluster of differentiation markers, B-cell acute lymphoblastic leukemia, Flow cytometry, Immunophenotyping

## INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a hematological malignancy characterized by the rapid proliferation of lymphoblasts, which are immature lymphoid cells.<sup>[1]</sup> Among the various subtypes of ALL, B-cell acute lymphoblastic leukemia (B-ALL) is the most common, particularly affecting children.<sup>[1,2]</sup>

The prognosis of B-ALL is influenced by several critical factors. Age at diagnosis is a significant determinant, with children aged 1–10 years generally exhibiting a better prognosis compared to infants and adults.<sup>[3]</sup> The presence of specific immunophenotypic markers can indicate different subtypes of B-ALL, which may respond variably to treatment. Moreover, certain genetic abnormalities, such as the presence of the *BCR-ABL1* fusion gene, are associated with a poorer prognosis, highlighting the importance of genetic profiling in the management of B-ALL.<sup>[4]</sup>

The research conducted by Wimalachandra *et al.* in 2020 on B-ALL in Sri Lanka revealed several key findings.<sup>[5]</sup> Approximately 80% of patients diagnosed with ALL were identified as having B-ALL. A significant proportion of these cases occurred in children, particularly those aged 2–6 years, indicating a peak incidence in this age group. High expression rates of specific B-cell markers were noted, including cluster of differentiation (CD19), which was present in nearly all cases, and CD10, detected in 93% of pediatric cases. CD22 was typically expressed in B-ALL, while TdT indicated the immaturity of lymphoblastic cells. The incidence of B-ALL was found to be more common in males, although the adult group showed a more balanced gender distribution.

The study also highlighted the presence of specific genetic abnormalities, such as the *BCR-ABL1* fusion gene, particularly in adults. Those patients commonly presented

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with symptoms of bone marrow failure, including fatigue, increased risk of infections, and bleeding tendencies.

Despite these findings, it is important to note that data related to B-ALL in Sri Lanka are still very limited, underscoring the need for further research to enhance understanding and improve management strategies for this condition.

Therefore, our study was also focused to describe the immunophenotypic features of a cohort of Sri Lankan patients diagnosed with B-ALL, utilizing flow cytometry to identify specific B-cell markers and assess their clinical implications.

## MATERIALS AND METHODS

This descriptive analytical study encompasses 47 cases of B-ALL diagnosed between June 2021 and December 2024.

The diagnosis of B-ALL was established based on the morphology of leukemic cells observed in bone marrow, and peripheral blood smears, along with immunophenotypic analysis conducted using multiparametric flow cytometer on bone marrow aspirates or peripheral blood samples.<sup>[6]</sup>

Patients currently undergoing treatment for B-ALL were excluded from participation. In addition, individuals with insufficient clinical information and other pertinent details were excluded from the study.

Peripheral blood or bone marrow samples used for this study were collected and sent to Sri Jayewardenepura General Hospital Laboratory for diagnostic purposes. Those samples were collected in EDTA anti-coagulated tubes. The analysis was performed using 8 color BD FACS Canto™ II Flow Cytometer, which was equipped with three lasers capable of detecting up to eight colors. The fluorochromes employed in the analysis included V450, V500c, FITC, PE, PerCP CY5.5, PE CY7, APC, and APC H7. The computer workstation was calibrated and maintained with quality control measures in place, and the results were analyzed using Becton Dickinson (BD) FACS Diva software.<sup>[7]</sup> The immunophenotypic analysis utilized the following markers: CD45, CD34, CD19, CD7, smCD3, cyCD3, cyMPO, cyCD79a, CD20, CD15, CD10, CD5, HLA-DR, CD64, CD13, CD117, CD33, and CD14.

The reagents used to detect expression of CD markers were selected following the guidelines and EuroFlow eight-color antibody panels for immunophenotyping of hematological malignancies and the validated protocols given by BD Bioscience, India.<sup>[7]</sup>

Blast cell identification was carried out using forward scatter versus side scatter (SSC) parameters, as well as CD45 intensity versus SSC-H dot plots. The fluorescence intensities of the blast cells were compared to those of the negative cell population to assess the expression of various CD markers.

A CD marker was considered positive if expressed by more than 20% of the gated population and dim positive expression was determined by 10–20% expression of the CD marker.<sup>[7]</sup>

## RESULTS

### Demographic data

The cohort included 2–82-year-old patients with a mean age of 35.1 years, representing significant adult representation. Fifteen pediatric cases (aged below 18 years) and 32 adult cases (aged over 18 years) of B-ALL were reported, and gender distribution was near even (51.1% female and 48.9% male), which is different from earlier findings of male predominance in pediatric B-ALL. Bone marrow samples represented 76.6% of cases, which is in line with standard diagnostic practice for B-ALL.

### Immunophenotypic characterization of B-ALL

Immunophenotypic profiling demonstrated consistent expression of core B-cell antigens [Table 1]. CD19, a pan-B-cell antigen, was positive in 100% of instances (47/47) with strong positivity (++/+) in 46 instances and one case of dim positive, confirming its diagnostic utility. CD10 (CALLA), a precursor B-cell antigen, was positive in 89.4% (42/47) of instances, though somewhat lower than the 93% in a former pediatric-focused Sri Lankan study.

This discrepancy is possibly because the current cohort also included adults. CD20, which is typically negative in immature B-ALL blasts, was negative in 57.4% (27/47) of the cases, also supporting the precursor B-ALL diagnosis. cyCD79a, a cytoplasmic B-lineage commitment marker, was positive in 93.6% (43/47) of the cases, confirming B-cell origin.

Markers of immaturity were prominent: TdT, a lymphoid immaturity marker, was positive in 66% (31/47) of cases, and CD34, a stem cell marker, was present in 97.9% (46/47) of cases, and HLA DR was positive in 100% (47/47).

### Diagnostic subtypes and aberrancies

The majority of the cases (53.2%, 25/47) were classified as typical B-ALL group without aberrant markers. Of

**Table 1:** Expression of B-cell lineage markers.

CD makers expression	CD marker			
	CD19	CD20	cyCD79a	CD10
++	38/47	4/47	18/47	28/47
+	8/47	11/47	25/47	11/47
Dim+	1/47	5/47	2/47	3/47
Negative	-	27/47	2/47	5/47

CD: Cluster of differentiation

those with aberrant markers, CD13+ and CD33+ B-ALL (12.8% each) were most frequent [Table 2]. There were rare combinations, including CD33+CD15+cyMPO+ (2.1%) and CD13+CD33+CD15+ (2.1%), that revealed phenotypic heterogeneity. These aberrances may reflect underlying genetic complexity, since myeloid marker expression in B-ALL typically is associated with poor prognostic factors such as BCR-ABL1 fusion or KMT2A rearrangement.

### Correlations and prognostic implications

There was no statistical correlation between CD45 blast percentage and age ( $r = 0.256$ ,  $P = 0.082$ ), and it was not significant. The presence of abnormal myeloid markers (e.g., CD13/CD33) and advanced age (median: 35.1 years) may be ominous in that adult B-ALL and myeloid-antigen-positive types are often associated with refractory disease. However, the absence of genetic or outcome data limits definitive prognostic conclusions.

### Comparison with prior Sri Lankan research

The findings partially align with the 2020 study by Wimalachandra *et al.*<sup>[5]</sup> While CD10 expression was slightly lower (89.4% vs. 93%), this may reflect the inclusion of adults, who often exhibit lower CD10 positivity. The balanced sex distribution in this cohort contrasts with the male predominance reported in pediatric B-ALL, underscoring age-related demographic variations. The high prevalence of CD34 (97.9%) and TdT (66%) aligns with global B-ALL profiles, reinforcing the utility of these markers in diagnosis.

The expression of various CD markers in adult and pediatric populations, highlighting the distribution of marker expression levels categorized as “++,” “+,” “DIM+,” and “NEGATIVE.”<sup>[8]</sup> Statistical significance (Asymp. sig) was assessed for each marker, with none reaching conventional significance levels ( $P < 0.05$ ), indicating that differences in expression between adult and pediatric groups may not be statistically significant.

## DISCUSSION

The analysis of B-ALL in our study, which encompassed 47 cases revealed significant insights into the immunophenotypic characteristics of this hematological malignancy. Notably, the mean age of patients was 35.1 years, indicating a substantial representation of adult cases, which contrasts with the traditional view of B-ALL predominantly affecting children. This finding aligns with the research conducted by Wimalachandra *et al.* (2020), which highlighted a peak incidence of B-ALL in children aged 2–6 years.<sup>[5]</sup> However, our study's broader age distribution underscores the necessity of considering adult cases in the overall understanding of B-ALL epidemiology.

Immunophenotypic profiling in our cohort demonstrated a high expression of core B-cell antigens, particularly CD19 and stem cell marker CD34, with positivity rates of 97.9%. These findings are consistent with global profiles of B-ALL, reinforcing the diagnostic utility of these markers. The expression of CD10 was slightly lower in our study (89.4%) compared to the 93% reported by Wimalachandra *et al.* This discrepancy may be attributed to the inclusion of adult patients in our analysis, who often exhibit lower CD10 positivity.<sup>[5,9]</sup> Furthermore, the presence of aberrant myeloid markers, such as CD13 and CD33, in subsets of patients (32% and 30%, respectively) suggests potential associations with high-risk genetic abnormalities. This observation is supported by previous studies, which indicated that myeloid marker expression in B-ALL is often linked to poor prognostic factors.<sup>[10,11]</sup>

The clinical implications of our findings on CD markers are significant. The elevated expression of CD34 and TdT reinforces their prognostic importance, as noted in prior research by Gujral *et al.*, 2009, and Paul *et al.*<sup>[11,12]</sup> The presence of aberrant myeloid markers in our cohort raises concerns regarding the underlying genetic complexities that may predict unfavorable outcomes.<sup>[13]</sup>

The presence of myeloid markers in our study aligns with the observations made by Larson (2006), who emphasized the challenges in managing adult B-ALL due to its often-aggressive nature.<sup>[14,15]</sup>

**Table 2:** Expression of aberrantly positive CD markers.

CD makers expression	Aberrant CD markers						
	CD13	CD33	CD15	CD7	cyMPO	CD2	CD5
++	3/47	3/47	1/47	-	-	-	-
+	4/47	8/47	4/47	2/47	-	1/47	-
Dim+	8/47	3/47	-	-	1/47	-	3/47
Negative	32/47	33/47	42/47	45/47	46/47	46/47	44/47

CD: Cluster of differentiation

In Shopsowitz *et al.*'s research in 2021, cyMPO and CD117-positive B-ALL cases demonstrated strong expression of CD33 but remained negative for CD10, thus demonstrating a distinct immunophenotypic profile that is related to certain leukemic subtypes.<sup>[16]</sup> These results are consistent with these observations, since we had one case of cyMPO dim positive B-ALL that was CD33 and CD117 positive but only dim positive for CD10. This particular immunophenotypic profile suggested a possible overlap in lymphoid and myeloid marker expression, reflecting a complicated differentiation status of the mixed phenotypic acute leukemia.

### Limitation

The relatively small sample size ( $n = 47$ ) and the lack of molecular data restrict the ability to draw definitive prognostic conclusions. Future research should aim to integrate cytogenetic analysis and stratify results by age to enhance diagnostic precision and prognostic stratification in Sri Lankan B-ALL patients. Expanding the cohort size and including genetic profiling will provide a more comprehensive understanding of the immunophenotypic heterogeneity and its implications for treatment outcomes.

**Data availability statement:** Data supporting these findings are available within the article or on request.

**Sample availability:** After analysis, blood and bone marrow specimens were discarded according to the guidelines given by the Infection Control Unit of the Sri Jayewardenepura General Hospital.

### CONCLUSION

Overall, the immunophenotypic heterogeneity among the cases of B-ALL in the cohort is defined as a broad collection of diverse cases with a significant implication for diagnosis and prognosis. The balanced gender distribution and access to pediatric and adult cases highlight the requirement for age-stratified examination of B-ALL studies. Elevated expression of large B-cell antigens CD19, cyCD79a, and CD34 validates their prognostic importance, while widespread expression of such abnormal myeloid antigens as CD13 and CD33 suggests underlying genetic complexities which may be predictive of unfavorable prognostic factors.

Despite the failure to detect statistically significant correlations of age with CD45 blast percentage, the data indicate that adult B-ALL cases, particularly those with expression of myeloid markers, are associated with more refractory disease. Together, these results emphasize the importance of precise immunophenotypic analysis in the appreciation of B-ALL heterogeneity and its prognostic implications, and the requirement for further genetic investigation to discern the associations with high-risk abnormalities.

**Author's contributions:** TB: Conceptualization, software, validation, investigation; TB & CF: Methodology, formal analysis; TB, CF, SM, PK, & CK: Resources; TB, CF, SM, & PK: Writing—original draft preparation; TB, CF, & PK: Validation; TB, PK, & CK: Writing—review and editing; TB & CK: Visualization, project administration; CK: Data curation. All authors have read and agreed to the published version of the manuscript.

**Ethical approval:** The research/study approved by the Institutional Review Board at Sri Jayewardenepura General Hospital, number SJGH/21/ERC/017, dated 25th June, 2021.

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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