Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.7197

CD66C EXPRESSION IN PEDIATRIC B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: CORRELATION WITH CD38, CD58, CD81, AND ITS ROLE IN MINIMAL RESIDUAL DISEASE DETECTION.

Dr. Santosh Suman[1*], Dr. Sangeeta Pahuja[2], Dr. Shailaja Shukla[3], Dr. Sunita Sharma[4], Dr. Jagdish Chandra[5], Dr. Alice Xalxo[6]

[1*]Department of Pathology, Lady Hardinge Medical College, Delhi, India. Email Id: santoshsuman_143@yahoo.com
[2]Department of Pathology, Lady Hardinge Medical College, Delhi, India, Email Id: sangeeta.pahuja@yahoo.com
[3]Department of Pathology, Lady Hardinge Medical College, Delhi, India, Email Id: drshailajalhmc@outlook.com
[4]Department of Pathology, Lady Hardinge Medical College, Delhi, India, Email Id: sunitasharma.123@outlook.com
[5]Department of Pediatrics, Lady Hardinge Medical College, Delhi, India, Email Id: drjagdish_lhmc@rediffmail.com
[6]Department of Pathology, Lady Hardinge Medical College, Delhi, India, Email Id: santoshsuman_143@yahoo.com

***Corresponding Author:** Dr. Santosh Suman
*Department of Pathology, Lady Hardinge Medical College, Delhi, India. Email Id: santoshsuman_143@yahoo.com

ABSTRACT

Background: This study aims to characterize the expression of CD66c in leukemic blasts of B-cell acute lymphoblastic leukemia (B-ALL) and evaluate its role in detecting Minimal Residual Disease (MRD).

Methodology: A hospital-grounded prospective observational investigation was done from November 2017 to March 2019 at Lady Hardinge Medical College along with Kalawati Saran Children Hospital, New Delhi, involving 30 patients diagnosed with B-ALL. Inclusion criteria were patients under 18 years, newly diagnosed with B-ALL. Exclusion criteria included patients with relapsed B-ALL or those who had received corticosteroids for ≥8 days prior. Routine investigations included a complete hemogram, and special investigations involved immunophenotyping by flow cytometry. MRD was defined as ≥0.01% leukemic cells.

Results: CD38 Expression: 96.67% (29/30) showed under expression; MRD positivity at day 35 was 91.66%. CD58 Expression: 90% (27/30) showed overexpression; MRD positivity at day 35 was 91.67%. CD81 Expression: All cases (25/25) showed expression; MRD sensitivity was 100%. CD66c Expression: 43.3% had <5% gated, 46.7% had >20% gated; dim expression in 60%, moderate in 40%. MRD sensitivity was 58.3%.

Conclusion: CD66c is a significant marker in B-ALL, but its sensitivity for MRD detection is lower compared to CD38, CD58, and CD81. The investigation highlights the importance of utilising multiple markers for effective MRD detection.

Keywords: B-cell acute lymphoblastic leukemia, CD66c, Minimal Residual Disease, flow cytometry, immunophenotyping

INTRODUCTION

Acute lymphoblastic leukemia (ALL) originates from genetic changes in lymphoid stem cells, leading to the uncontrolled growth of immature lymphoid cells. It primarily affects children under six, accounting for about 75% of cases [1]. Despite treatment advances, relapse remains a significant challenge and a leading cause of treatment failure. Lineage heterogeneity in ALL is characterized by lymphoid cells expressing markers typically associated with myeloid cells [2]. While myeloid antigens' abnormal expressionon ALL cells is extensively reported, it usually lacks prognostic or even therapeutic significance but can be valuable for MRD. MRD is the small count of leukemia cells remaining after treatment and serves as a powerful prognostic indicator in childhood [3] ALL. MRD detection allows physicians to assess relapse risk and tailor chemotherapy accordingly. Assessing myeloid antigens on ALL cells helps track treatment response and detect residual leukemia cells, indicating a higher relapse risk. While lineage heterogeneity may not directly impact prognosis or treatment decisions, its use in MRD monitoring provides valuable information for guiding patient management and improving outcomes [4]. Although flow cytometry analysis is a widely utilized and cost-effective way for MRD detection, current markers may not adequately identify MRD amidst normal B-cell precursors, contributing to false negativity rates[5].

Leukemia-associated immunophenotype (LAIP) presents a promising approach for identifying novel biomarkers in MRD assessment. In India, research on CD66c expression in ALL and its role in MRD is limited. CD66c, or Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6), is a GPI-linked protein mainly found in granulocytes and their precursors. In pediatric B-ALL, CD66c expression correlates with markers like CD38, CD58, and CD81, offering insights into the molecular profile of B-ALL [6]. These correlations can refine diagnostic and prognostic strategies, such as a positive correlation between CD66c and CD38 or CD58 indicating common biological mechanisms or disease phenotypes. CD66c is also significant for MRD detection, crucial for predicting relapse risk and tailoring chemotherapy [5] . Flow cytometry analysis of CD66c provides a fast, reliable method for MRD detection, aiding in disease monitoring and therapeutic adjustments. Overall, studying CD66c expression in pediatric B-ALL enhances understanding of disease pathogenesis and improves MRD detection, leading to better patient outcomes. This investigation looks to probe the expression patterns of CD66c in leukemic blasts of B-cell acute lymphoblastic leukemia (B-ALL) and to explore its significance in the detection of MRD.

METHODOLOGY

Study Design:

A hospital-based follow-up analytical prospective observational study was done at the Department of Pathology, and Lady Hardinge Medical College, along with the Department of Pediatrics, Kalawati Saran Children Hospital, New Delhi, spanning from November 2017 to March 2019. The sample population consisted of 30 patients diagnosed with Acute Lymphoblastic Leukemia.

Selection criteria

Inclusion Criteria:

Patients younger than 18 years old who were newly diagnosed cases of B-cell ALL and were registered and willing to undergo treatment at Lady Hardinge Medical College, along with the Department of Pediatrics, Kalawati Saran Children Hospital, New Delhi,

Exclusion Criteria:

- Patients having relapsed B-cell ALL.
- Patients who got corticosteroids for ≥8 days prior to enrollment in the study.

Procedure

Routine Investigations: Complete hemogram: hemoglobin, differential leukocyte count, total leukocyte count, platelet count, and peripheral smear analysis using Sysmex XN-1000.
Special Investigations: Immunophenotyping by Flow Cytometry using Beckman Coulter FC500.

Sample Collection and Processing:

- Peripheral blood collection for complete blood count, cytochemistry, peripheral smear, and immunophenotyping.
- Bone marrow aspirate or peripheral blood samples processed for flow cytometry analysis using Beckman Coulter Cytomics FC500 within 24-48 hour

Technique Used for the study

- Flow Cytometry: Flow cytometry measured fluorescence (Beckmann Coulter FC500) and optical characteristics of single cells using antibodies conjugated to fluorescent dyes. Multiple fluorochromes were used to measure various cell properties simultaneously.
- MRD Detection: MRD was detected by flow cytometry, defining MRD positivity as ≥0.01% leukemic cells in the total population of nucleated cells. Complete morphologic remission was defined as <5% blasts morphologically in the bone marrow aspirate, and/or lack of blasts inside the CSF and/or testes of regular size and echotexture at the induction phase's end.

Data Analysis: Data were recorded in MS Excel and analyzed using SPSS v21. Descriptive statistics, chi-squared tests, independent sample t-tests, Pearson's correlation coefficient, and Cohen's kappa values were configured. The significance level was set at $p < 0.05$.

RESULTS

In 30 cases of B-cell ALL, 28 were CALLA positive (CD10+), and 2 were CALLA negative (CD10-). The patients' ages ranged - 1.5 to 12 years, having a mean of 4.8 years along with a standard deviation of 2.83. The majority (11 cases) were aged 2 to 4 years. No cases were found in children under 1 year or over 12 years. So, the male-to-female ratio was 2:1 (which was 20 males, 10 females), with males accounting for 66.66% of cases. Of the two CALLA negative cases, one was male, and one was female.

TABLE 1: CASE DISTRIBUTION (n=30)

Type	Frequency	Percentage
CALLA Positive	28	93.3%
CALLA Negative	2	6.7%

Table 1 shows that out of 30 B-cell ALL cases, 93.3% were CALLA positive (CD10+) and 6.7% were CALLA negative (CD10-).

TABLE 2: CD38 expression in lymphoblasts at diagnosis (n=30)

CD38: % Gated	Frequency	Percentage
<5%	0	0.0%
5 – 10%	0	0.0%
10 – 15%	0	0.0%
15 – 20%	1	3.33%
>20%	29	96.67%

CD38 Expression	Frequency	Percentage
Dim	29	96.67%
Moderate	1	3.33%
Bright	0	0.0%

As shown in table 2 among all the cases, 96.67% had CD38 gated >20%, and 3.33% had CD38 gated 15-20%. Regarding CD38 expression, 96.67% were dim, 3.33% were moderate, and none were bright.

TABLE 3: CD58 expression in lymphoblasts at diagnosis (n=30)

CD58: % Gated	Frequency	Percentage
<5%	0	0.0%
5 – 10%	0	0.0%
10 – 15%	0	0.0%
15 – 20%	0	0.0%
>20%	30	100.0%
CD58 Expression	Frequency	Percentage
Dim	3	10%
Moderate	27	90%
Bright	0	0.0%

As Shown in Table 3 all cases (100%) had CD58 gated >20%. CD58 expression was dim in 10% of cases and moderate in 90%, with no bright expression observed at day 0.

TABLE 4: CD66c expression in lymphoblasts at diagnosis (n=30)

CD66c: % Gated	Frequency	Percentage
<5%	13	43.3%
5 – 10%	2	6.7%
10 – 15%	1	3.3%
15 – 20%	0	0.0%
>20%	14	46.7%
CD66c Expression	Frequency	Percentage
Dim	18	60.0%
Moderate	12	40.0%
Bright	0	0.0%

As shown in table 4 CD66c expression, 43.3% had <5% gated, 6.7% had 5-10% gated, 3.3% had 10-15% gated, and 46.7% had >20% gated. CD66c expression was dim in 60% of cases and moderate in 40%, with no bright expression at day 0.

TABLE 5: CD81 expression in lymphoblasts at diagnosis (n=25)

CD81: % Gated	Frequency	Percentage
<5%	0	0.0%
5 – 10%	0	0.0%
10 – 15%	0	0.0%
15 – 20%	0	0.0%
>20%	25	100.0%
CD81 Expression	Frequency	Percentage
Dim	4	16.0%
Moderate	19	76.0%
Bright	2	8.0%

As shown in Table 5 CD81 was assessed in 25 out of 30 cases. All 25 cases had CD81 gated >20%. CD81 expression was dim in 16% of cases, moderate in 76%, and bright in 8% at day 0.

TABLE 6: Expression of Immunological Marker at Day 35 for Detecting the Presence of Minimal Residual Disease (MRD)

Marker Expression		MRD POINT	
		N	%
CD 38	>0.01&	11	91.7%
	<0.01%	1	8.3%
CD58	>0.01&	11	91.7%
	<0.01%	1	8.3%
CD66c	>0.01&	7	58.3%
	<0.01%	5	41.7%
CD 81	>0.01&	12	100.0%
	<0.01%	0	0.0%

As shown in Table 6 at Day 35, the expression of immunological markers was assessed for detecting Minimal Residual Disease (MRD). For CD38 and CD58, 91.7% of cases showed MRD when the expression was greater than 0.01%, while only 8.3% showed MRD with expression below 0.01%. For CD66c, 58.3% of cases had MRD when expression exceeded 0.01%, compared to 41.7% below 0.01%. Interestingly, all cases with CD81 expression above 0.01% were positive for MRD, while none were detected when expression was below 0.01%.

TABLE 7: Sensitivity of Immunological Marker Expression at Day 35 for Detecting the Presence of Minimal Residual Disease (MRD)

Marker	Sensitivity
CD38	91.7%
CD58	91.7%
CD66c	58.3%
CD81	100.0%

As demonstrated in Table 7 about the sensitivity of immunological markers for detecting Minimal Residual Disease (MRD) at Day 35 varied across markers. CD38 and CD58 showed a sensitivity of 91.7%, indicating their effectiveness in detecting MRD. CD66c exhibited a slightly lower sensitivity at 58.3%. In contrast, CD81 demonstrated the highest sensitivity among the markers, with all cases showing 100.0% sensitivity for MRD detection.

Pearson's correlation coefficient revealed strong positive correlations across CD38 and CD58 (0.969, $p < 0.000$), CD38 and CD81 (0.887, $p < 0.000$), CD81 and CD58 (0.973, $p < 0.000$), and CD66c and CD123 (0.973, $p < 0.000$). Moderate positive correlations were discovered across CD66c and CD81 (0.605, $p = 0.037$) and CD123 and CD81 (0.606, $p = 0.037$). However, there were minimal to weak, statistically non-significant correlations between CD66c and CD38, as well as CD66c and CD58.Kappa level of agreement analysis demonstrated statistically significant ($p < 0.001$) agreement (kappa 1.000) between CD38 and CD58, CD38 and CD123, and CD58 and CD123 in detecting MRD at day 35. However, CD66c did not exhibit statistically significant agreement with other MRD markers.

DISCUSSION

This investigation's discoveries underscore the critical role of CD66c expression in pediatric B-ALL and its potential utility in MRD detection. CD66c, a glycosylphosphatidylinositol (GPI)-linked protein, emerged as a significant marker when assessing MRD, contributing to the broader understanding of leukemic cell profiles and aiding in precise treatment strategies.

In our study, at day 35, CD38 under expression was observed in 11 out of 12 MRD-positive cases (91.66%), with a sensitivity of 91.7% for detecting MRD using CD38 LAIP. Similarly, Verbeek et al.,(2024) [6] reported CD38 LAIP expression in 95 out of 103 pre-B cell ALL/biphenotypic leukemia cases (92%), including 65 children. Kara et al.,(2021) [7] found CD38 underexpression in 60 out of 73 newly diagnosed precursor B ALL cases (82.19%).
Also our study, show CD58 on day 35, CD58 overexpression was seen in 11 out of 12 MRD-positive cases (91.67%), with a sensitivity of 91.7% for MRD detection using CD58 LAIP. Similarly, Kulis et al.,(2022)[8]reported CD58 overexpression in 92 out of 103 pre-B cell ALL/biphenotypic leukemia cases (89%), including 65 children, aligning with our findings. However, Rocha et al.,(2019)[9]found CD58 overexpression in 43 out of 73 newly diagnosed precursor B ALL cases (58.9%), which is lower than our reported rate.
Another finding of our study shows CD81 at day 35, 11 out of 12 MRD-positive cases (91.66%) showed moderate CD81 expression, and 1 case (8.33%) showed dim expression. The sensitivity of CD81 LAIP for detecting MRD was 100%. Gaipa et al.,(2018)[10] reported that in 98 pre-B-ALL cases analysed at diagnosis or even relapse, CD45 dim leukemic blasts had dropped CD81 expression inside 80 cases (82%). Hendrick et al.,(2019)[11] found that in 85 out of 86 B-ALL cases, hematogones and lymphoblasts could be distinguished utilising a combination of CD45, and CD19, and CD34, and CD10, and CD20, and CD38, and CD58, and CD81.
Our results demonstrate significant CD66c expression in leukemic blasts of B-ALL patients, aligning with previous studies that identified CD66c as the most frequently observed aberrant myeloid marker in B-cell precursor ALL. Correlation analysis revealed notable relationships between CD66c and other markers such as CD38, CD58, and CD81, particularly with CD38 and CD81. These correlations suggest a potentially shared pathophysiological mechanism or disease phenotype, emphasizing the importance of comprehensive immunophenotyping in understanding leukemia's molecular underpinnings.

Similarly, Mejstrikova et al.,(2017) [4]. reported myeloid antigen expression in 57.4% of childhood B-ALL cases, with CD13 being the most common marker (46.3%), and 7 B-ALL cases expressing T antigen CD7. Salem et al. reported aberrant expression of CD33 inside 10.5% of cases and CD13 inside 7.9% of B-ALL cases. Virk et al.,(2023)[5] found that aberrant myeloid antigen expression was more common in B-ALL (36%) than in T-ALL.
One of the key outcomes of this study is establishing CD66c's utility in MRD detection. Flow cytometry incorporating CD66c effectively identified residual leukemic cells, enhancing MRD detection’s specificity and sensitivity when combined with other markers. This improvement is crucial for stratifying patients based on relapse risk and tailoring therapeutic regimens. Similarly, Pierzynar et al.,(2021) [3] reported CD66c expression in 81.4% of newly diagnosed B-ALL patients, more frequently than CD13, CD33, CD17, or CD123. For MRD detection (≥0.01% positive cutoff), CD66c demonstrated 85.3% sensitivity, 86.2% specificity, 84.2% positive predictive value, 87.2% negative predictive value, and 85.8% accuracy.
In summary, this study confirms the significance of CD66c expression in pediatric B-ALL and its crucial role in enhancing MRD detection. The correlations observed with other immunological markers underscore the complexity of leukemic cell biology and the necessity of comprehensive diagnostic approaches.

CONCLUSION:

This study demonstrates that CD66c is a significant marker in B-ALL, contributing to the immunophenotypic profile of leukemic blasts. However, its sensitivity for detecting MRD is lower compared to other markers such as CD38, CD58, and CD81. The discoveries propose that while CD66c can be a useful marker, the use of a multi-marker approach, incorporating CD38, CD58, and CD81, enhances the accuracy and reliability of MRD detection in B-ALL patients. This multi-marker

strategy is essential for optimizing treatment protocols and improving prognostic assessments in paediatric B-ALL.

REFERENCES

1. Juárez-Avendaño, G., Méndez-Ramírez, N (2021). Molecular and cellular markers for measurable residual disease in acute lymphoblastic leukemia. *Boletín médico del Hospital Infantil de México*, *78*(3), 159-170.
2. Lebecque, B., Besombes, J., Dannus, L. T., (2024). Faster clinical decisions in B-cell acute lymphoblastic leukaemia: A single flow cytometric 12-colour tube improves diagnosis and minimal residual disease follow-up. *British Journal of Haematology*.
3. Pierzyna-Świtała, M., Sędek, Ł., Kulis, J., Mazur, B., (2021). Multicolor flow cytometry immunophenotyping and characterization of aneuploidy in pediatric B-cell precursor acute lymphoblastic leukemia. *Central European Journal of Immunology*, *46*(3), 365-374.
4. Mejstríková, E., Hrusak, O., Borowitz, M. J., (2017). CD19-negative relapse of pediatric B-cell precursor acute lymphoblastic leukemia following blinatumomab treatment. *Blood cancer journal*, *7*(12), 659.
5. Virk, H., & Sachdeva, M. U. S. (2023). Flow Cytometric MRD Assessment in Acute Lymphoblastic Leukemias. *Indian Journal of Medical and Paediatric Oncology*.
6. Verbeek, M. W., & van der Velden, V. H. (2024). The Evolving Landscape of Flowcytometric Minimal Residual Disease Monitoring in B-Cell Precursor Acute Lymphoblastic Leukemia. *International Journal of Molecular Sciences*, *25*(9), 4881.
7. Kárai, B., Tisza, K., Eperjesi, O., Nagy, A. C., (2021). A Novel Method for the Evaluation of Bone Marrow Samples from Patients with Pediatric B-Cell Acute Lymphoblastic Leukemia—Multidimensional Flow Cytometry. *Cancers*, *13*(20), 5044.
8. Kulis J, Sędek Ł, Słota Ł, Perkowski B,. Commonly assessed markers in childhood BCP-ALL diagnostic panels and their association with genetic aberrations and outcome prediction. Genes. 2022 Jul 31;13(8):1374.
9. Rocha, J. M. C., Xavier, S. G., de Lima Souza, (2019). Current strategies for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. *Mediterranean journal of hematology and infectious diseases*, *8*(1).
10. Gaipa, G., Buracchi, C., & Biondi, A. (2018). Flow cytometry for minimal residual disease testing in acute leukemia: opportunities and challenges. *Expert Review of Molecular Diagnostics*, *18*(9), 775-787.
11. Hendricks, C. L., Buldeo, S., Pillay, D., Naidoo, A., (2019). Comparing morphology, flow cytometry and molecular genetics in the assessment of minimal residual disease in children with B-acute lymphoblastic leukaemia (B-ALL). *South African Journal of Oncology*, *3*, 8.