International Journal of Innovative Research in Medical Science (IJIRMS)
Volume 10, Issue 05, May 2025,
https://doi.org/10.23958/ijirms/vol10-i05/2075

Original Article

Role of CD86 in Detection of Minimal Residual Disease in Pediatric B-cell Acute Lymphoblastic Leukemia: A Prospective Study of 40 Pediatric Patients

Dr. Riva Choudhary [1], Dr. Smita Singh *[1], Dr. Rushali Saxena [2], Dr. Shailaja Shukla [3], Dr. Deonath Mahto [4],

[1]Department of Pathology, Lady Hardinge Medical College, New Delhi, India.
[2]Senior Resident, Department of Pathology, Lady Hardinge Medical College, New Delhi, India.
[3]Director Professor, Department of Pathology, Lady Hardinge Medical College, New Delhi, India.
[4]Director Professor, Department of Pediatrics, Lady Hardinge Medical College, New Delhi, India.

*Corresponding Author: Dr. Smita Singh, MBBS, MD; smitasingh2556@gmail.com

Received: 18 April 2025; Accepted: 05 May 2025; Published: 12 May 2025

Abstract

Objective: This study aims to evaluate to utility of CD86 in minimal residual disease (MRD) detection in paediatric B-cell Acute Lymphoblastic Leukemia (ALL) for better patient management. ***Materials and Methods:*** Clinical data and flowcytometric analysis of B-cell ALL on day 0 and day 35 for MRD was collected prospectively from paediatric haematology oncology centres in India. ***Results:*** 40 children newly diagnosed with B-cell ALL between for MRD detection were enrolled in the study. Frequency of CD73, CD86 and CD123 were 90%, 70% and 60% respectively in diagnostic sample. In MRD positive samples, maximum frequency of CD86 (85.7%) and CD73 (85.7%) were observed followed by CD123 (71.4%). CD86 showed highest post-induction stability among all Leukaemia Associated Immunophenotype (LAIP) markers on blasts. ***Conclusion:*** CD86 has a significant expression in B-cell ALL in MRD detection with excellent post induction stability. Thus, inclusion of CD86 improves the utility of flowcytometry based routine clinical practice of MRD detection.

Keywords: ***CD86, Acute lymphoblastic leukemia, Minimal residual disease, flowcytometry.***

Introduction

Minimal Residual Disease (MRD) remains a considerable barrier to the prognosis and outcome of Acute Lymphoblastic Leukaemia (ALL) [1]. Notwithstanding the multitude of treatment protocols accessible, leukaemia relapse continues to be a significant contributor to treatment failure in patients with B-cell ALL [2,3].

Diverse methodologies, including Multiparametric Flow Cytometry (MFC) and molecular techniques such as Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) and Next-Generation Sequencing (NGS), are employed to assess Minimal Residual Disease (MRD) [4].

MFC is regarded as a great laboratory technique because to its speed, simplicity, cost-efficiency, and extensive accessibility [5]. Nonetheless, MFC encounters a hurdle in detecting MRD in a small percentage of patients due to either the expression of a restricted number of Leukaemia Associated Immunophenotype (LAIP) markers on blasts or the deletion of LAIP markers resulting from drug-induced immunomodulation [6].

CD86 significantly contributes to anti-tumor immunity by functioning as a costimulatory molecule. It results in the inhibition of both cell-mediated and humoral immune responses, facilitating immunological evasion by the developing tumor [7]. Leukemic patients exhibiting elevated levels of CD86 demonstrate worse prognostic outcomes [8].

The current study seeks to emphasize the incorporation of CD86 into immunophenotyping techniques alongside CD73, CD123, CD81, CD38, and CD58 to enhance the sensitivity of minimal residual disease diagnosis. This research may provide significant insights to the field and assist in the formulation of more effective prognostic and management techniques for paediatric B-cell ALL.

Methods and Materials

This study comprised 40 newly diagnosed paediatric instances of B-cell acute lymphoblastic leukaemia (ALL). The diagnosis of B-cell acute lymphoblastic leukaemia (ALL) was established based on morphology, cytochemistry, and immunophenotypic antigen expression according to WHO 2017 criteria. Patients underwent

therapy according to the ICiCLe protocol [9] established by the Department of Paediatrics at Kalawati Saran Children Hospital in New Delhi. LAIP indicators were examined in Bone Marrow (BM) or peripheral blood at the commencement of treatment (Day 0). A bone marrow examination was conducted on Day 35 post-induction to assess minimal residual disease (MRD). The institutional review board has sanctioned the study, and informed consent has been acquired from patients, their parents, or their guardians.

Multicolour Flow Cytometric Immunophenotyping

Procedure: A volume of 100 µl of bone marrow material was aliquoted into a test tube. The pre-titrated volume of antibodies was added and blended using vortexing. The tubes were incubated in darkness at ambient temperature for 20 minutes. The tubes were incubated for 20 minutes following the addition of 500 µl of Optilyse. The tubes underwent centrifugation at 1500 rpm for 5 minutes, after which the supernatant was discarded. The particle was fragmented and resuspended in 4000 µl of sheath fluid. After vortexing, the tubes underwent centrifugation at 1500 rpm for 5 minutes. After the last wash, the pellet was resuspended in 500 µl of sheath fluid. The sample was obtained using a pre-calibrated flow cytometer (Beckman Coulter Cytomics FC 500). A minimum of 100,000 events per tube were gathered for diagnostic immunophenotyping.

Analysis of Outcome: Sequential gating was employed for all the tubes. Antibody combinations in which leukemic blasts occupied vacant areas, separate from regions containing normal B cell progenitors, were recognised as valuable Leukaemia Associated Immunophenotypes (LAIP). A threshold exceeding 10% of gated blasts was applied for all antigens. In all instances, autofluorescence was eliminated using the unstained control tube from the acute leukaemia panel. Samples exhibiting >0.01% of leukemic blasts expressing at least two LAIP markers or one immaturity marker in conjunction with a LAIP marker were classified as MRD positive.

Statistics

Data were coded and recorded in MS Excel spreadsheet programme. Data analysis was performed using software SPSS v21. Descriptive statistics was elaborated in the form of mean, median and standard deviations for continuous variables, and frequencies and percentage for categorical variables. Group comparisons were made using independent sample t-test for continuously distributed data, and chi squared test for categorical data. Spearman correlation coefficient was calculated for LAIP markers. Level of statistical significance was taken as $p<0.05$.

Results

Expression of LAIP markers on Leukemic Blasts at Diagnosis (Day 0) and following post-induction chemotherapy (Day 35)

On Day 0, Blast exhibited overexpression of CD86, CD73, and CD123 in 70%, 90%, and 60% of cases, respectively, alongside the expression of dim CD38 and moderate to bright CD58 in 97.5% of cases (**Fig.1A**).

At the Day 35 MRD assessment, 14 cases (35%) were positive for MRD, exhibiting overexpression of CD86, CD73, and CD123 in 85.7%, 85.7%, and 71.4%, respectively. In MRD positive cases, the expression of Dim CD38 and moderate to bright CD58 was observed in 92.8% of cases. (**Fig.1B**) (**Table 2**).

The Spearman rank correlation coefficient indicated a statistically significant association of CD86 with CD73 (1.00) and Dim CD38, as well as a moderate to bright correlation with CD58 (1.00). No significant association was detected between CD86 and CD123.

CD86

Stability of LAIP markers post-induction in MRD-positive cases on Day 35

All LAIP indicators demonstrated robust post-induction stability in MRD-positive subjects on Day 35 compared to the Day 0 immunophenotype. The peak stability on day 35 was noted, with loss of expression occurring in only one MRD positive case, characterised by CD86 expression and dim CD38 alongside moderate to bright CD58 levels. No gain of 86 was reported in any MRD-positive individual (**Table 3**).

Comparison of CD86 with clinical haematological parameters and NCI risk classifications

CD86 was not significantly associated with age, gender, clinical symptoms and organomegaly on Day 0 and Day 35. (**Table 4**) CD86 had a significant association with lymphadenopathy on Day 0 ($p=0.049$). CD86 had no significant association with hematological parameters hemoglobin, total leukocyte counts over 50,000/µl, thrombocytopenia below 50,000/µl, and blast percentage exceeding 50% on Day 0 and Day 35. At diagnosis, 8 of 14 (57.14%) MRD-positive cases were categorized as intermediate risk, while 6 of 14 (42.85%) were classed as high risk. MRD detection was substantially higher (40%) in the CD86 Positive intermediate risk category compared to the CD86 Negative intermediate risk group (0%) ($p=0.046$). In the high-risk group, MRD detection was observed to be higher in the CD86 positive cohort (80%) compared to the CD86 negative cohort (40%) ($p=0.197$) (**Table 5**).

Table 1: The MRD antibody panel

TUBE NO.	FITC	PE	ECD	PC5	PC7
MRD Tube 1	CD38	CD58	CD34	CD10	CD19
MRD Tube 2	CD45	CD123	CD34	CD10	CD19
MRD Tube 3	CD20	CD14	CD13	CD10	CD19
MRD Tube 4	CD45	CD73	CD34	CD10	CD19
MRD Tube 5	CD45	CD86	CD34	CD10	CD19

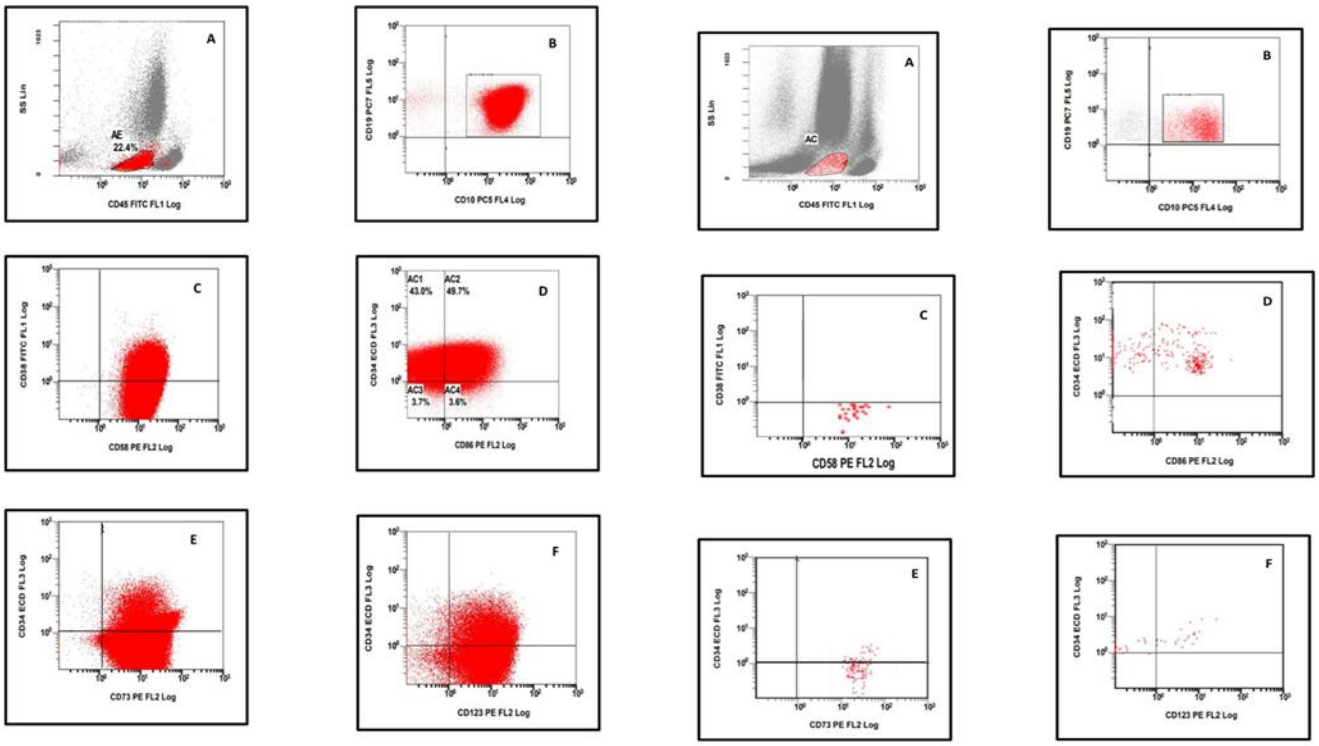

Fig1: This figure displayed CD45 vs low SSC gated dot plots demonstrating LAIP markers on B cell ALL blast. A. At Day 0 CD19 and CD10 co-expressing blast showed expression of CD73, CD86 and CD123. B. At Day 35 post-induction residual blast demonstrated expression of CD73, CD86 and CD123

Table 2: Expression of LAIP markers on Leukemic Blasts at Diagnosis (Day 0) and on post-induction chemotherapy (Day 35)

	DAY 0 (n=40)	DAY 35 (MRD positive n=14)
CD86	28	12
CD73	36	12
CD123	24	10
Dim CD38 With Moderate to Bright CD58	39	13

Table 3: Post-Induction Stability of Markers in MRD Positive Cases at Day 35 (n=14)

Markers	Day 0	Day 35	Gain of LAIP	Loss of LAIP
CD86	13/14(92.8%)	12/13(92.3%)	-	1/14(7.14%)
CD73	14/14(100%)	12/14(85.7%)	-	2/14(14.2%)
CD123	11/14(78.5%)	10/11(90.9%)	1/14(7.14%)	2/11(18.2%)
Dim CD38 with Moderate to Bright CD58	14/14(100%)	13/14(92.8%)	-	1/14(7.14%)

Table 4: Comparison of CD86 with clinical parameters at day 0 (n=40) and in MRD positive cases on Day 35 (n=14)

Parameters	D0 CD86 +	D0 CD 86 -	Total	P value	D35 CD86 +	D35 CD 86 -	Total	P value
Age>10 year	8(72.7%)	3(27.3%)	11	0.817	5(100%)	-	5	0.255
Gender-Male	18(69.3%)	8(30.7%)	26	0.885	7(87.5%)	1(13.5%)	8	0.825
Fever	24(70.5%)	10(29.5%)	34	0.847	8(88.9%)	1(11.1%)	9	0.649
Weight loss	9(75%)	3(25%)	12	0.651	6(85.7%)	1(13.5%)	7	1.000
Bleeding	9(81.8%)	3(27.2%)	11	0.817	5(100%)	-	5	0.255
Headache	1(50%)	1(50%)	2	0.527	1(50%)	1(50%)	2	0.119
Joint pain	0	1(100%)	1	0.122	0	1(100%)	1	0.011
Hepatomegaly	21(65.6%)	11(34.4%)	32	0.227	10(83.3%)	2(16.7%)	12	0.533
Splenomegaly	21(67.7%)	10(32.3%)	31	0.563	10(83.3%)	2(16.7%)	12	0.533
Lymphadenopathy	14(58.33%)	10(41.66)	24	0.049	8(80%)	2(20%)	10	0.334
Hb <8	21(67.7%)	10(32.3%)	31	0.563	10(83.3%)	2(16.7%)	12	0.533
TLC >50,000	7(58.3%)	5(41.6%)	12	0.292	5(100%)	0	5	0.255
Platelets <50000	24(68.6%)	11(31.4%)	35	0.602	10(83.3%)	2(16.7%)	12	0.672
Blasts >50	26(70.2%)	11(29.8%)	37	0.896	12(83.3%)	2(16.7%)	14	-

Table 5: MRD Detection in CD86 positive and CD86 negative B-cell ALL cases based on NCI Risk Category

NCI Risk Category	D0 CD86 positive B-cell ALL	MRD detection in CD86 positive B- cell ALL	D0 CD86 negative B- cell ALL	MRD detection in CD86 negative B- cell ALL	P value
Standard Risk	3	0(0%)	0	0(0%)	-
Intermediate Risk	20	8(40%)	7	0(0%)	0.046
High Risk	5	4(80%)	5	2(40%)	0.197

Discussion

B-cell ALL is a clonal haematologic malignancy originating from B-lymphoid progenitor cells [10]. CD86 is a novel LAIP marker that has not been thoroughly investigated previously. These investigations indicate that CD86 overexpression may serve as a significant biomarker for therapy monitoring and disease prognostication [7,10].

Prior research has emphasised the significance of CD86 on leukemic blasts, primarily facilitating IgG isotype release to circumvent immune monitoring and sustain blast survival through elevated expression of anti-apoptotic molecules and reduced levels of pro-apoptotic molecules [10]. These methods indicate that elevated CD86 levels confer a survival advantage to leukemic blasts by obstructing programmed cell death [8].

The objective of this study was to assess the expression of CD86 and other LAIP markers on B cell ALL blasts in children and their relevance in MRD monitoring.

In the current investigation, at day 0, the overexpression of LAIP markers was most pronounced with Dim CD38, accompanied by moderate to bright CD58 (92.8%), followed by CD73 (90%), CD86 (70%), and CD123 (60%). CD73 and CD86 exhibited results analogous to those of Coustan-Smith *et al.*, Tembhare *et al.*, Sedek *et al.*, and Jain *et al.* (2,6,12-13) Jain *et al.* and Coustan-Smith *et al.* noted a higher incidence of CD123 overexpression compared to CD86 [2,13].

On day 35 following induction chemotherapy, the prevalence of LAIP markers was highest for dim CD38, exhibiting moderate to bright expression of CD58 (92.8%), followed by CD73 (90%), CD86 (70%), and CD123 (60%). The overexpression of CD73 and CD86 was analogous to that seen by Tembhare *et al.* [6] The study by Sedek *et al.* [12] also demonstrated comparable expression levels of CD73 and CD86 in blasts on day 15 of treatment.

The post-induction stability of all MRD markers in our investigation was predominantly observed with Dim CD38, exhibiting moderate to high levels of CD58 (92.8%) and CD86 (92.3%), followed by CD123 (90.9%) and CD73 (85.7%). Tembhare *et al.* [6] noted the highest post-induction stability of CD86 among various LAIP markers. Sedek *et al.* [12] also noted good post-induction stability of CD86, after CD73. Consequently, the superior post-induction stability of CD86 as MRD indicators documented in prior research aligns with our findings.

The study by Mansour A *et al.* revealed that, after a follow-up period of 28 months, 16 patients had recurrence and exhibited elevated CD86 expression compared to those who maintained complete remission. Consequently, they determined that elevated levels of CD86 may correlate with unfavourable prognosis [8]. Our investigation noted a higher incidence of MRD positivity in CD86-expressing blasts at day 0 compared to the CD86-negative group, potentially indicating the prognostic significance of CD86 in B-cell acute lymphoblastic leukaemia, as proposed by Mansour A *et al.* [8] Wahba *et al.* discovered a statistically significant correlation between CD86 expression and adverse outcomes in these patients[14].

This study demonstrates the relevance of CD86 as a valuable LAIP marker for MRD, exhibiting strong post-induction stability.

Study Limitations

Smaller number of cases were enrolled due to limited period of study and resource constraints. A large cohort of study is recommended to substantiate out findings.

Conclusion

Notwithstanding current protocols and sophisticated therapies, the recurrence of leukaemia continues to be a significant challenge. The early identification of remaining blasts, undetectable by cytomorphological examination, is crucial for attaining full remission. CD86 is becoming a possible biomarker for minimal residual disease identification with strong post-induction stability in B-cell acute lymphoblastic leukaemia. Therefore, we propose inclusion of CD86 in the flow cytometric panel for minimal residual disease detection to enhance the accuracy of MRD identification in B-cell acute lymphoblastic leukaemia.

Declarations

Authors' contributions

All the authors have read and approved the final manuscript.

Data Availability

Can be accessed on request from the corresponding author.

Funding Statement

None

Acknowledgments

None

Conflict of interest

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

[1] Cheng YQ, Zhai XW. Clinical application of minimal residual disease detection in childhood acute leukemia. Chinese Journal of Contemporary Pediatrics. 2018;20(5):416-20.

[2] Coustan-Smith E, Song G, Clark C, Key L, Liu P, Mehrpooya M *et al.* New markers for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2011;117(23):6267-76.

[3] Gaipa G, Basso G, Biondi A, Campana D. Detection of minimal residual disease in pediatric acute lymphoblastic leukemia. Cytometry B Clin Cytom. 2013;84(6):359-69.

[4] Fuda F, Chen W. Minimal/measurable residual disease detection in acute leukemias by multiparameter flow cytometry. Curr Hematol Malig Rep. 2018;13(6):455-66.

[5] Panda SS, Radhakrishnan V, Ganesan P, Rajendranath R, Ganesan TS, Rajalekshmy KR, *et al.* Flow cytometry based MRD and its impact on survival outcome in children and young adults with ALL: A prospective study from a tertiary cancer centre in southern India. Indian J Hematol Blood Transfus. 2020;36(2):300-8.

[6] Tembhare PR, Ghogale S, Ghatwai N, Badrinath Y, Kunder N, Patkar NV *et al.* Evaluation of New Markers for Minimal Residual Disease Monitoring in B- Cell Precursor Acute Lymphoblastic Leukemia: CD73 and CD86 Are the Most Relevant New Markers to Increase the Efficacy of MRD Assay. Cytometry Part B. 2016;94(1):100-11.

[7] Suvas S, Singh V, Sahdev S, Vohra H, Agrewala JN. Distinct Role of CD80 and CD86 in the Regulation of the Activation of B Cell and B Cell Lymphoma. J Biol Chem. 2002;277(10):7766-75.

[8] Mansour A, Elkhodary T, Darwish A, Mabed M. Increased expression of costimulatory molecules CD86 and sCTLA-4 in patients with acute lymphoblastic leukemia. Leukemia & Lymphoma. 2014;55(9):2120-4.

[9] Zuckerman T, Rowe JM. Pathogenesis and prognostication in acute lymphoblastic leukemia. F1000Prime Rep. 2014;6:59.

[10] Das, N., Banavali, S., Bakhshi, S. *et al.* Protocol for ICiCLe-ALL-14 (InPOG-ALL-15-01): a prospective, risk stratified, randomised, multicentre, open label, controlled therapeutic trial for newly diagnosed childhood acute lymphoblastic leukaemia in India. Trials 23, 102 (2022).

[11] Hock B, Patton W, Budhia S, Mannari D, Roberts P, Mckenzie J. Human plasma contains a soluble form of CD86 which is present at elevated levels in some leukaemia patients. Leukemia. 2002;16(5):865-73.

[12] Sedek L, Theunissen P, Costa ESD, Sluijs-Gelling AVD, Mejstrikova E, Gaipa G *et al.* Differential expression of CD73, CD86 and CD304 in normal vs. leukemic B-cell precursors and their utility as stable minimal residual disease markers in childhood B-cell precursor acute lymphoblastic leukemia. J of Immunol Methods. 2018; pii: S0022- 1759(17):30126-6.

[13] Jain S, Mehta A, Kapoor G, Bhurani D, Jain S, Agrawal N *et al.* Evaluating New Markers for Minimal Residual Disease Analysis by Flow Cytometry in Precursor B Lymphoblastic Leukemia. Indian J Hematol Blood Transfus. 2018;34(1):48-53.

[14] Wahba MA, El-Maraghy HM, Abdel-Razik AM. CD86: A Novel Prognostic Marker in Acute Lymphoblastic Leukemia Patients. The Egyptian Journal of Hospital Medicine. 2018;71(6): 3373-7.