 DOI: 10.18231/2394-6792.2017.0122

Clinical Significance of CD34 & CD10 Expression in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Introduction: Prognostication of acute leukemia by detecting immunophenotypic marker expression on leukemic cells by flow cytometry is crucial in providing individualized treatment and improving outcome in Pediatric ALL (Acute lymphoblastic leukemia). The Aim of the study was to determine whether expression of CD34 and CD10 could be used as additional prognostic markers in predicting treatment response in Pediatric ALL cases.

Materials and Method: This study was conducted among 44 pediatric ALL cases diagnosed in 2 year period in a tertiary care centre in North Kerala. Peripheral smear (PS), & bone marrow (BM) were examined, clinical and laboratory parameters were studied and flow cytometry was done in all cases and expression of CD10 & CD34 were noted. Treatment response was assessed by examining peripheral smear on day 7 after treatment, BM after induction, Cerebrospinal fluid (CSF) examination during induction phase .All the cases were followed up.

Result: In our study it was observed that presence of blasts on day 7 peripheral smear and CSF were more in CD34 negative cases compared to CD34 positive cases. BM remission after induction, treatment response and good outcome on follow up were more among CD34 positive cases. The results were statistically significant for CD34 expression. CD10 expression alone was not found to be statistically associated with treatment response.

Conclusion: CD34 expression predicts treatment response and is a significant favourable prognostic factor in pediatric ALL cases. It can be included in risk stratification for planning treatment along with established parameters.

Keywords: Pediatric ALL, CD34, CD10 flow cytometry, Treatment response

Introduction

Acute leukemia is the most common hematological malignancy worldwide which continues to be the largest contributor to cancer related mortality in children.(1) About 60-85% of all leukemias reported are acute lymphoblastic leukemia (ALL). Two most likely reasons for high mortality in acute leukemia for resource poor nations are lack of enough criteria for monitoring and ongoing evaluation and lack of categorization of patients in to risk groups and giving category specific individualized treatment. The diagnosis and classification of leukemia rely on the simultaneous application of multiple techniques, morphology combined with cytochemistry and multi-parameter flow cytometry. The immunophenotypic classification of ALL is based on the surface marker expression of leukemic blasts and recognizes two lineages of lymphocytes T and B cells.(2)

Flow cytometry is a modern technique which can bring out molecular features of malignant cells and provide valuable clues for deciding treatment at relevant stages.(3) Immunophenotyping of cases of ALL (Acute lymphoblastic Leukemia) is a very important application of Flow cytometry and provides an easy and definite diagnosis and a distinction between B-cell ALL (B-ALL) and T-cell ALL (T-ALL) and also for sub classification.(4) Cell surface phenotype in lymphoblastic leukemia have variably found to have prognostic significance.(5) CD10 & CD34 are surface markers that have been reported to have prognostic relevance in childhood acute lymphoblastic leukemia, but the results were conflicting. The initial response to therapy is a strong predictor of outcome, patients with reduction in peripheral blast count after 7 day induction prophase have more favorable prognosis.(6)

Aim of the study

To study the role of CD34 and CD10 expression or their co-expression in predicting treatment response in Pediatric Acute Lymphoblastic leukemia

Materials and Method

The study was conducted among 44 pediatric ALL cases diagnosed during a 2 year period in a tertiary care centre in North Kerala. Diagnosis was made according to the WHO criteria by examining peripheral smear, bone marrow, cytochemistry with myeloperoxidase (MPO), periodic acid Schiff (PAS) and by doing flow cytometry. They were treated in the department of pediatrics at the same centre during which Peripheral Smear at day 7 of starting treatment, BM at day 28 (Bone Marrow after induction), were examined in all cases. CSF cytology for blasts was done at beginning of treatment. Cases were clinically followed up during the course of treatment for a period of 2 years during which follow up peripheral smears and bone marrows were

examined to look for relapse. Institutional Ethics Committee approval and informed consent of the patient/guardian were obtained.

Flow cytometry: Samples taken were either blood or BM depending on total WBC count and blast count. Flow cytometry was done using 4 color flow cytometer model BD FACS Calibur with fluorochrome antibodies using standard protocols. Flurochromes used for antibodies were FITC (Fluroscein isothiocyanate), APC (Allophycocyanin), PerCP (Peridinium Chlorophyllate) and PE (Phycoerythrin). The primary panels of antibodies were used to distinguish AML (Acute Myeloid leukemia) from ALL and to further classify ALL into B- ALL and T- ALL. Antibodies were either surface or cytoplasmic. Light scattering due to laser light reflecting and refracting off the intersecting cells. – 2 types, **Forward scatter** (FS)-light that gets scattered away along axis of the beam which correlated with cell size, **Side scatter (SS**) - light scattering at wide angle, which correlated with granularity/cellular complexity.[7] Gating was based on either forward or side scatter characteristics or on CD45/ side scatter characteristics.[8]

Primary panel of anti-bodies

CD19, CD10- B lineage
CD7, CD5-T lineage
CD13, CD33, CD117 -Myeloid
CD34, HLADR- stem cell marker
TdT- early lymphoid marker

Secondary panel of anti-bodies

Cytoplasmic CD3- marker of T cell lineage
Cytoplasmic CD79a- marker of B cell lineage
MPO- marker of myeloid differentiation

Fluorochromes and antibodies

APC- CD117, HLADR, TDT, cCD3
FITC- CD10, CD33, Cd7, MPO
PerCP- CD45, CD19
PE- CD34, CD13, CD5, cCD79a

Procedure: Flow cytometry was done according to BD FACS Calibur manufacturer instructions. 2ml blood/bone marrow collected in K+ EDTA vacationer. 100 microlitre of blood/bone marrow sample pipetted into FACS tube followed by antibodies in order. (For APC fluorochrome - 2 micro liters, for all other flurochromes 5 micro liters of antibodies added). Maximum of 4 antibodies were used in a tube depending on the fluorochrome. CD 45 added to all the tubes for CD45 gating. The tubes were incubated at room temperature, then 2 ml of a lysing solution added in 1/10 dilution for lysing RBCs, then the tubes were vortexed, incubated, again vortexed & then centrifuged. 2ml sheath fluid added for hydrodynamic focusing which helped cells to align in a single file in direction of flow. The tubes were then centrifuged, supernatant discarded and run in flow cytometer. For cytoplasmic antibodies a permibilizing solution for permeabilizing cell membrane also added.

Interpretation: Marker expression was interpreted as positive or negative by analysing intensity of antigen expression, that is the antigen binding capacity of a given fluorochrome antibody conjugate. The first log decalog (between 10^0 and 10^1) represented negative fluorescence and signals falling in the second, third and fourth decaloges represented weak/dim (+), moderate (++), and strong/bright (+++) fluorescence intensity, respectively. Cases were classified as B-ALL and T-ALL depending on marker expression. The expression of CD3 and CD10 were specifically looked for.

CSF: CSF was examined for blasts by cytocentrifugation in a cytospin.

Risk stratification and treatment: Modified BFM (Berlin-Frankfurt-Munich) protocol was used for treatment. Patients were classified as standard risk or high risk.[9] The risk was taken as **standard risk** when age was between 1 year and 10 years, total count <50000, morphological type L1or L2, immunophenotype B ALL and cytogenetics showed t(12;21) or hyperploidy. **High risk** included cases with age of <1 and >10 years, WBC count of >50000, L3 morphology, T-ALL, hypodiploidy, t(9;22) or t(4;11), mediastinal widening, CNS involvement and blasts on day 7 of starting treatment. The treatment included Preinducion, Induction, reinduction, and maintenance phases. Remission was described by microscopic criteria, <5% blasts in a cellular marrow, recovery of peripheral neutrophils and platelets, and absence of detectable extramedullary leukemia. Leukemic infiltration of CNS was defined as 5 or more leukocytes/mm^3 and blast cells in CSF or cranial nerve palsy which is a well-established prognostic factor in children with ALL.[10]

Poor response to treatment was indicated by the presence of day 7 blast, BM not in remission after induction & CNS involvement. The cases were divided in to 2 groups**: good outcome group,** which achieved remission at the end of induction with no relapse during follow-up and **poor outcome group** which included cases which were not in remission at the end of induction, those who died during induction phase and relapsed case.

Appropriate statistical analysis was done to analyze results.

Result

The age of children ranged from 2 years to 11 years (mean 5.1). 39 cases (88.6%) were in 1-9 age group and 5 (11.4%) were in the group either <1 or >10 years. Out of 44, 23cases (52.3%) were males and 21 (47.7%) were females.

26 cases (59.1%) were high risk and 18 (40.9%) were standard risk. ALL -L2 was more (70.5%) compared to ALL-L1 (29.5%). The peripheral smear, bonemarrow & PAS positivity in PS of a case of ALL L-2 are shown in Fig. 1. Out of 44 cases, 34 cases (77.3%) were B -ALL, 10 cases (22.7%) were T-ALL. Flow

cytometry of a case of B-ALL is shown in Fig. 2 and a case of T-ALL is shown in Fig. 3.

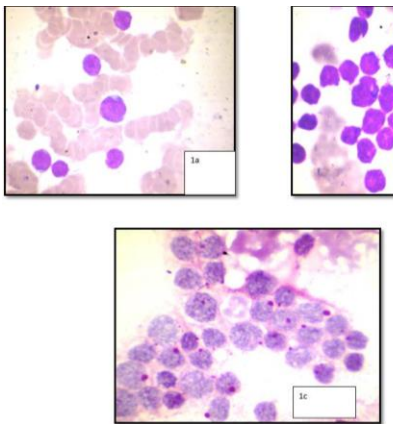

Fig. 1: a – peripheral smear 100x leishman stain, ALLL2, b: Bone marrow aspirate(100x)Leishman stain ALL-L2, c: Peripheral smear (100x) PAS stain block positivity in ALL-L2

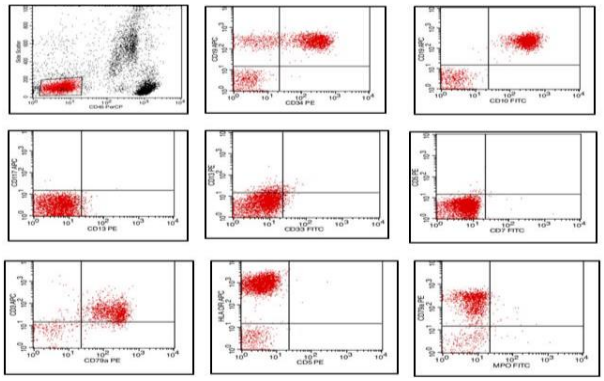

Fig. 2: flow cytometry in a case of B-ALL-CD45 gated cells comprise 33.89% of total 10000 events. CD 45 gated blasts are strongly positive for CD10, CD19, CD34, CD79a and HLA DR. Blasts are negative for CD13, CD33, CD117 and MPO, CD3, CD5

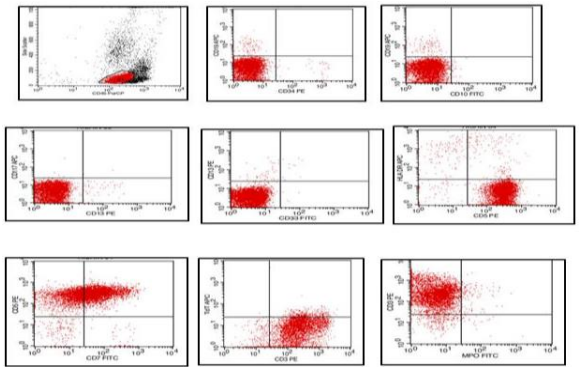

Fig. 3: Flow cytometry in a case of T-ALL CD45 gated cells comprise 70.24% of total 10000 events. CD45 gated cells are strongly positive for T cell markers - cCD3, CD5, CD7, negative for CD10, CD19, CD13, CD33, CD34, CD117, TdT & HLA-DR

Blast in Day 7 Peripheral smear was detected in 16 (36.4%) cases. CSF blast was positive in 10cases (22.7%). Seven patients died (15. 9%) during induction phase. Bone marrow after induction was examined in remaining 37 cases. 32 cases out of 37 (86.5%) were in remission and 5 cases (13.5%) were not in remission. On follow up, 1 out of 32 cases relapsed (3.1%). The presence of day 7 blast, CSF blast and BM response after induction were analyzed for association with type of ALL and CD34 and CD10 expression (Table 1, 2, 3 respectively).

Table 1: day 7 blast vs. immunophenotypic markers

	Day 7 blast + (16)	Day7 blast- (28)	Chi Square	p value
	n (%)	n (%)		
B-Lineage ALL (34)	9 (26. 5)	25 (73. 5)	6. 32	0. 012
T-Lineage ALL (10)	7 (70. 0)	3 (30. 0)		
CD10+ (32)	9 (28. 1)	23 (71. 9)	3. 44	0. 064
CD10- (12)	7 (58. 3)	5 (41. 7)		
CD34+ (34)	8 (23. 5)	26 (76. 5)	10. 6	0. 001
CD34- (10)	8 (80. 0)	2 (20. 0)		
CD34+CD10+ (29)	6 (20. 7)	23 (79. 3)	9. 03	0. 003
CD34&/orCD10- (15)	10 (66. 7)	5 (33. 3)		

Table 2: CSF blast vs Immunophenotypic markers

	n (%)		Chi square	P value
	CSF blast+ (10)	CSF blast- (34)		
Immunophenotyping B-Lineage ALL (34) T-Lineage ALL (10)	 7 (20. 6) 3 (30)	 27 (79. 4) 7 (70)	0. 39	0. 53
CD10 expression CD10+ (33) CD10- (11)	 7 (21. 2) 3 (27. 3)	 26 (78. 8) 8 (72. 7)	0. 04	0. 6
CD34 expression CD34+ (34) CD34- (10)	 5 (14. 7) 5 (50)	 29 (85. 3) 5 (50)	5. 4	0. 01
Combination of CD10 and CD34 CD10+CD34+ (29) CD10 &/or CD34- (15)	 5 (17. 3) 5 (33. 3)	 24 (82. 7) 10 (66. 7)	1. 4	0. 22

Table 3: BM day28 vs. Immunophenotype

	BM Day 28 in remission (32)	BM Day 28 not in remission (5)	chi square	p value
	n (%)	n (%)		
Immunophenotyping B-Lineage ALL (29) T-Lineage ALL (8)	 27 (93. 1) 5 (62. 5)	 2 (06. 9) 3 (37. 5)	5. 025	0. 020
CD10 expression CD10+ (27) CD10- (10)	 25 (92. 6) 7 (70)	 2 (7. 4) 3 (30)	3. 18	0. 07
CD34 expression CD34+ (30) CD34- (7)	 28 (93. 3) 4 (57. 1)	 2 (06. 7) 3 (42. 9)	6. 3	0. 012
Combination of CD10 and CD34 CD10+CD34+ (25) CD10 &/or CD34- (12)	 24 (96. 0) 8 (66. 6)	 1 (04. 0) 4 (33. 4)	5. 9	0. 015

Table 4: Outcome vs. Immunophenotype

	Good outcome (31)	Poor outcome (13)	Chi square	p value
	n (%)	n (%)		
B-Lineage ALL (34)	26 (76. 4)	8 (23. 6)	2. 6	0. 1
T-Lineage ALL (10)	5 (50. 0)	5 (50. 0)		
CD10+ (33)	24 (72. 7)	9 (27. 3)	0. 281	0. 5
CD10- (11)	7 (63. 6)	3 (27. 4)		
CD34+ (34)	27 (79. 4)	7 (20. 6)	5. 7	0. 01
CD34- (10)	4 (40. 0)	6 (60. 0)		
CD34+CD10+ (29)	23 (79. 3)	6 (20. 7)	3. 2	0. 07
CD34&/CD10- (15)	8 (53. 3)	7 (46. 7)		

Table 5: Risk stratification vs. expression of CD34 and co-expression of CD34, CD10

Risk	CD34+	CD34-	p value	CD34+ CD10+	CD34&/or CD10-	p value
	n (%)	n (%)				
SR (18)	17 (94. 4)	1 (5. 6)	**0. 02**	17 (94. 4)	1 (5. 6)	**<0. 001**
HR (26)	17 (65. 4)	9 (34. 6)		12 (46. 2)	14 (53. 8)	

Table 6: Immunophenotype of ALL vs expression of CD34 and co-expression of CD34 and CD10 in pediatric ALL (n=44)

Type	CD34+	CD34-	p value	CD34+CD10+	CD34&/or CD10-	p value
B-ALL(34)	31(91.2)	3(8.8)	<0.001	29(85.3)	5(14.7%)	<0.001
T-ALL(10)	3(30)	7(70)		0	10(100%)	

Among CD34 negative cases, 8 (80%) showed blasts on day 7 peripheral smear where as only 8 out of 23 (23.5%) CD34 positive cases showed blast positivity which was statistically significant. Also in cases which showed combined expression of CD34 and CD10, day 7 peripheral smear blast was detected only in 6 out of (20.7%) cases. Day 7 blasts were detected more among T-ALL compared to B-ALL. (Table 1). CSF blasts were detected more in CD34 negative cases (50%) compared to CD34 positive cases (14.7%) which showed statistical significance (Table 2). Out of 30 cases which showed CD34 positivity, 28 cases (93.3%) were in remission when bone marrow was examined after induction compared to (57.1%) CD34 negative cases. (Table 3). Out of 25 cases which showed combined expression of CD34 and CD10, 24 cases (96%) were in remission which was statistically significant(Table 3). 92.6% of CD10 positive cases were in remission. Bone marrow remission were more among B-ALL(93.1%) compared to T-ALL (62.5%). Good outcome group (which included 31 cases) & poor outcome group (which included 13 cases (7 deaths, 5 not in remission, 1 relapse) were analyzed for association with CD34 expression and co-expression of CD34 and CD10 expression. Results showed that good outcome group were more among CD34 positive cases (79.4%) compared to CD34 negative cases (40%) which was statistically significant. (Table 4). Among cases which showed co-expression of CD34 & CD10, 79.3% were in good outcome group. 60% of poor outcome group were CD34 negative.

Discussion

In this study, B-ALL outnumbered T-ALL which is comparable to other studies. In this study death occurred during initial induction phase of treatment. Other studies also reported the same.[11] CD34 expression and co-expression of CD34 and CD10 were more among the SR group and among B- ALL. The percentage of CNS involvement was more compared to other studies.

In our study, absence of Day 7 peripheral smear blast & absence of CSF blast, BM remission after induction were more in cases which showed either CD34 expression or co expression with CD10. All these showed statistically significant association with CD34 expression. The good outcome group also showed association with CD34 expression. CD34 expression was more among SR cases (Table 5) and also among B-ALL cases (Table 6). Out of 18 SR cases, 17 (94.4%) were CD34 positive (Table 5). When separately analyzed for B-ALL, 31 out of 34 cases were CD34+, with absence of day 7 blast & remission after induction more among CD34 + cases. 32 out of 34 cases were CD10 positive and 29 cases showed co-expression (Table 6). The study conducted by Cascavilla et al[12] and Pui et al[13] showed that CD34 was frequently expressed in B-lineage ALL and was a positive prognostic factor with early remission and absence of CNS leukemia. A study by Eddy Supriyadi[14] et al in Indonesian children concluded that expression of CD10 alone or combined expression of CD34 and CD10 was associated with favorable outcome in children. The study of Consolini et al[15] concluded that in their patient cohort CD10 expression in B and T lineage ALL had no independent prognostic significance. In our study among T-ALL, 3 cases were CD34+ out of which 2 were in remission after induction. Only 1 case was CD10 positive and none of the cases showed co-expression of CD34 & CD10, which was in contrast to the Indonesian study and the study by Dakka[16] et al. In our study CD10 expression alone was not found to be statistically associated with the treatment response, so the association of co-expression of CD34 and CD10 may be due to the contributory effect of CD34. Being short period study, overall survival was not calculated in this study, instead surrogate markers like presence of day 7 blast, BM remission, CSF blast were used to predict treatment outcome .In future similar studies for determining role of surface marker expression on overall survival and event free survival can be done.

Conclusion

CD 34 expression predicts treatment response in pediatric ALL. CD34 & B-lineage are independently favorable prognostic indicators. CD 10 expression alone was not found to be statistically significant. CD34 expression was found to be more among standard risk group and among B-ALL. CD34 is a promising candidate to be included in the risk stratification for planning treatment along with established parameters in pediatric ALL.

Acknowledgments

The authors thank the patients and their families for their support. We would also like to thank staff of departments of pathology and pediatrics.

Conflict of Interest

The authors declare no conflict of interest

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