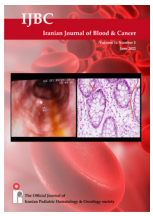
Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir



Original article

Significance of Cross Lineage Antigen Expression in Acute Lymphoblastic Leukaemia

Sindhura LK Laxminarayana[1], Aradhana Harrison[2], Phaneendra VR Datari[1], Sushma V Belurkar[1]

[1]Department of Pathology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, KA, India.
[2]Department of Pathology, Melaka Manipal Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, KA, India.

ARTICLE INFO

Article History:
Received: 7/6/2022
Accepted: 12/8/2022

Keywords:
Acute lymphoblastic leukaemia
Cross lineage
Aberrant antigen expression
Multiparametric flow cytometry
Immunophenotyping

***Corresponding authors:**
Dr. Aradhana Harrison
Assistant Professor, Department of Pathology, Melaka Manipal Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, KA, India.
Email: aradhanaharrison@gmail.com

Abstract

Background: Aberrant expression of cross-lineage antigens gives valuable insight into the diagnosis and prognosis of acute leukaemia. In countries like India, cytogenetic tests are widely accessible. Exploring the prognostic value of an accessible test is of great importance. Therefore, establishing a population-specific immunophenotype database will enable to design an antibody panel equipped to detect cross-lineage antigen expression. The aim of this study was to determine the frequencies of cross-lineage antigen expression in Acute Lymphoblastic Leukaemia (ALL), its relationship with clinical features, and changes in blood counts during the treatment course.

Methods: This was a retrospective observational study conducted at a tertiary care hospital. Consecutive ALL cases over 2 years were reviewed. Relation of cross-lineage aberrant antigens with blood counts and clinical features were studied. Chi-square test and Fisher's exact test were used.

Results: A total of 149 ALL cases were included in the study. Thirty (20.1%) cases showed expression of cross-lineage antigens. CD7 was the most commonly expressed cross-lineage antigen, seen in 14 (10.5%) cases of B-ALL. CD13, seen in eight (5.3%) patients, was the most frequent aberrant myeloid antigen. Myeloid aberrancies were associated with lower WBC count and blast count while aberrancies of T-cell antigens on B-ALL showed higher WBC count and blast count.

Conclusions: Cross lineage antigenic aberrancies influence blast count and WBC count. Documentation of these aberrancies in ALL helps in prognostication and monitoring of the disease.

Please cite this article as: LK Laxminarayana S, Harrison A, VR Datari P, V Belurkar S. Significance of Cross Lineage Antigen Expression in Acute Lymphoblastic Leukaemia. IJBC 2022; 14(3): 22-31.

1. Introduction

Acute Lymphoblastic Leukaemia (ALL) is a clonal hematopoietic stem cell malignancy. World Health Organisation incorporates antigenic expression and genetics for the sub-classification of ALL into B-cell ALL (B-ALL) and T-cell ALL (T-ALL) [1]. There is no specific morphologic feature that distinguishes B and T lymphoblasts [2]. Multiparametric flow cytometry helps in the evaluation of lineage-specific antigens and the detection of leukaemia-associated immunophenotype [3,4].

Diagnostic dilemma occurs when a single clustered cell population shows co-expression of antigens of more than one lineage. This is known as cross-lineage antigen expression [3,5,6]. It is a qualitative attribute accredited to 'lineage infidelity' and 'lineage promiscuity'. Lineage infidelity is the manifestation of an abnormal genetic constitution because of leukaemogenesis, and lineage promiscuity is due to the expansion of a physiological multipotent progenitor. Both these mechanisms are poorly understood and invoke enigma in areas of research [5].

The clinical significance and prognosis of cross-lineage antigen expression remain controversial [7,8]. A few studies have shown that cross-lineage myeloid antigen expression in T-ALL has higher rates of induction failure while other studies have shown no such association [9,10]. Further, some studies have shown that ALL patients expressing myeloid aberrancies have a poor response to therapy, while others have negated this inference [8,11,12,13]. Other studies have shown that the absence of myeloid antigen expression in ALL is associated with low platelet counts in children which makes them prone to bleeding [7]. Seemingly, no consensus has been reached on the implications of cross-lineage aberrant expressions. The World Health Organisation recognizes cross-lineage antigen expression as a key feature for the diagnosis of leukemic blasts which also provides useful clues to underlying cytogenetic and molecular abnormalities [14].

Diagnosis and prognostication of ALL based on the World Health Organisation classification require expensive cytogenetic and molecular tests available only in referral laboratories. On the other hand, immunophenotyping by multiparametric flow cytometry has gained popularity in India. Therefore, it can be used as a surrogate for testing for specific cytogenetic and molecular abnormalities, if the clinical and prognostic significance of cross-lineage antigen expression is known. Based on the presence of these aberrancies, a cost-effective diagnostic algorithm can be developed [7,15,16]. Most of the research on phenotypic aberrancies in acute leukaemia is from western literature. As racial and environmental factors affect leukaemogenesis, the frequency of cross-lineage antigen expression may be different in different geographies. Therefore, establishing a population-specific immunophenotype database will enable to design a comprehensive antibody panel specifically equipped to detect cross-lineage antigen expression. This study aimed to compare the frequencies of cross-lineage antigen expression in adult and paediatric ALL and to observe their relationship with clinical features at presentation and blood counts during the treatment course.

2. Material and Methods

This was a retrospective observational study conducted on cases of ALL from August 2016 to August 2018. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was reviewed and approved by the institutional committee (IEC513-2018). Informed consent was obtained from all patients as a part of the in-patient admission procedure.

2.1 Patient recruitment:

Patients' details were retrieved from the laboratory information system. Consecutive cases of B-ALL and T-ALL between August 2016 and August 2018 which have multiparametric flow cytometry data of complete acute leukaemia panel of antibodies, treated at a tertiary care centre with standard chemotherapy regimen and have at least 6 months of follow-up, were included in the study. Cases of Acute Myeloid Leukaemia or Acute Leukaemia of Ambiguous Lineage and/or cases treated elsewhere and referred to our institute for follow-up were excluded from the study.

2.2 Clinical details and complete blood count parameters

Clinical details were collected from hospital records. Blood cell counts at diagnosis, at the end of induction, and during the maintenance phase were retrieved from the laboratory information system.

Ethylenediaminetetraacetic acid anticoagulated peripheral whole blood samples were analyzed on Beckman Coulter UniCel® DxH 800 and DxH 600 haematology analyzers. Differential leucocyte count was performed manually on Leishman-stained peripheral smears. Bone marrow was examined for cellularity, trilineage haematopoiesis, and dyspoiesis. Manual all nucleated cell differential count was performed. Quality control protocols were as per the Standard Operating Procedure of the laboratory.

2.3 Immunophenotyping:

Immunophenotyping reports of patients were reviewed concerning leukaemia associated immunophenotype and pattern of antigenic expression. Multiparametric flow cytometry had been performed on peripheral blood and/or bone marrow aspirate at diagnosis (treatment naïve). Flow cytometric acquisition and analyses were performed using BD FACSCantoTM II Flow Cytometer with 3 lasers with 4-3-3 combination and BD FACSDivaTM Software version 8.0.3.

Cytometer settings and instrument optimization was done using laboratory standards. Application settings were performed using BD OneflowTM setup beads. Staining of markers was performed by the standardized protocol. Samples were processed such that each tube had a cell concentration of one million cells per 100 microlitres. In four primary tubes, cells were stained with an eight-colour antibody panel (Supplementary S1). The monoclonal antibodies were conjugated to fluorochromes which included fluorescein isothiocyanate (FITC), allophycocyanine (APC), APC H7, phycoerythrin (PE), PE Cyanin 7 (PE Cy 7), peridinin chlorophyll protein cyanin 5.5 (PerCP Cy5.5), V450 and V500. All reagents were obtained from Becton Dickinson Biosciences, USA.

The diagnosis of ALL and cross-lineage antigen expression was based on the World Health Organisation 2008/2016 criteria.[14] After doublet discrimination and debris exclusion, all viable events were plotted against Cluster of Differentiation (CD)45 and side scatter area (SSC). Events expressing low to moderate CD45 and low side scatter were gated as blasts. B-cell lineage was assigned when blasts had an expression of CD19 with more than one of the following: cytoplasmic CD79a, CD10, or CD20. T-lymphoblasts were suspected when blasts had bright CD45 expression and low side scatter. T-cell lineage was assigned if the blasts expressed cytoplasmic or surface CD3.

A surface marker was considered positive when more than 20% of the gated events expressed the antigen, while a cytoplasmic marker was considered positive when more than 10% of the gated events showed antigen expression [17]. The blasts were considered positive for cytoplasmic Myeloperoxidase when >3% of gated events were positive. Intra-tube controls (normal B cells, normal T cells, neutrophils, and monocytes) and unstained controls were used to define a positive and negative expression for each marker (Supplementary S2).

2.4 Statistical methods:

The relationship between the expression of markers and clinical features were analyzed by the Chi-square test and Fisher's exact test. Quantitative data were expressed as mean along with standard deviation (mean±SD) and median, and the distribution of quantitative variables was determined using the Kolmogorov-Smirnov test. Mean values of quantitative variables were compared using the independent samples t-test data (for data with normal distribution) and Mann-Whitney test (for skewed data). A p-value of <0.05 was considered statistically significant.

3. Results:

A total of 149 patients of ALL were included in the study.

3.1 Clinical details and complete blood count parameters

The age of patients ranged from one to 70 years. The median age among paediatric patients was 4.5 years and among adults was 36 years. The overall male-to-female ratio was 1.32:1. B-ALL was more common than T-ALL. The distribution of patients in each category with range, mean and median of blast count, white blood cell count, and platelet count at diagnosis is shown in **Table 1**. Fever was the most common presenting feature. Hepatomegaly and splenomegaly were common in B-ALL, while mediastinal mass and lymphadenopathy were common in T-ALL. The spectrum of clinical features with frequency is shown in **Figure 1**.

Table 1. Distribution of patients in each category with white blood cell count, platelet count and blast count at diagnosis in Acute Lymphoblastic Leukaemia

		B-ALL		T-ALL	
		Adult B-ALL [n=59(39.5%)]	Pediatric B-ALL [n=74(49.6%)]	Adult T-ALL [n=7 (4.6%)]	Paediatric T-ALL [n=9 (6.0%)]
White blood cell (x10^9/litre)	Range	0.3 - 112	0.9 – 215	1.5 – 205	39.6 - 460
	Mean	20.4	20.3	74.1	250
	Median	10.45	8.65	57.3	273
Platelets (x10^9/litre)	Range	5 – 279	6.0 – 302	16 – 304	8 – 439
	Mean	50.64	59.7	89.3	85.3
	Median	152	36	32	39
Blasts (%)	Range	0 – 94	0 – 88	0 – 86	30 – 99
	Mean	47.5	36.4	44.5	78.1
	Median	71.5	33	45	84
Total			**n=133 (89.2%)**		**n=16 (10.7%)**

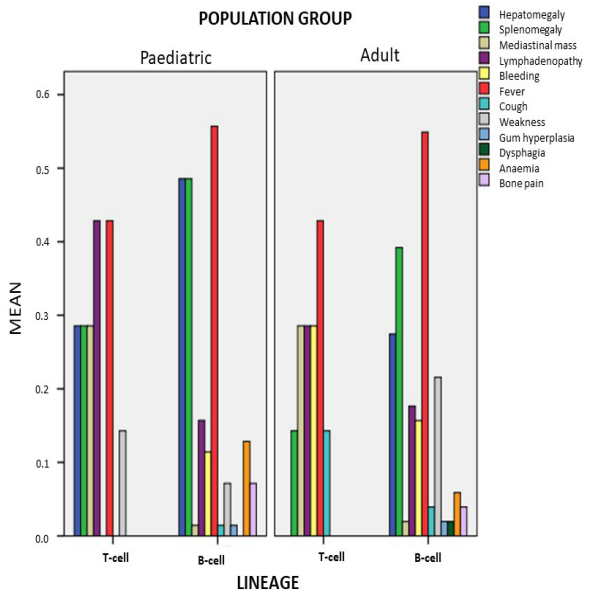


Figure 1: Clinical features in Acute Lymphoblastic Leukaemia in adult and paediatric patients .

3.2 Immunophenotyping by multiparametric flow cytometry:

Out of the total 149 cases of ALL, 30 (20.1%) showed cross-lineage aberrant expression. CD13 was the most frequent myeloid antigen that was expressed in ALL. Aberrant CD7 expression was the most common T-cell antigen expressed in B-ALL. The frequency of cross-lineage antigen expression is shown in **Table 2**.

and their distribution among adult and paediatric patients is shown in **Figure 2**.

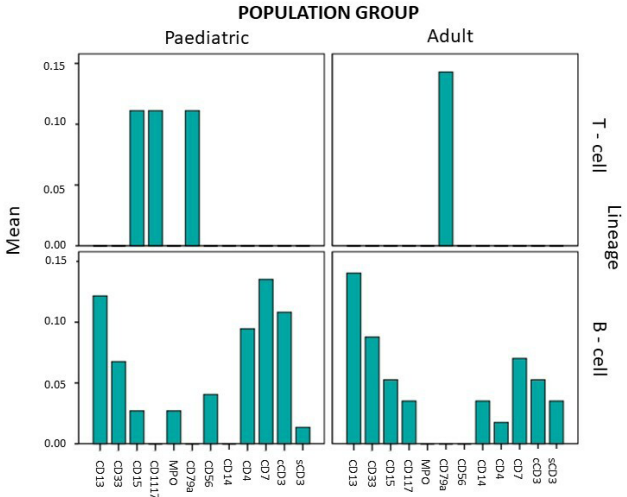


Figure 2: Aberrant antigens in Acute Lymphoblastic Leukaemia - Frequencies among adult and paediatric patients

3.3 Relationship of cross lineage antigen expression with complete blood count parameters and clinical features at diagnosis

At diagnosis, the mean blast count, white blood cell count, and platelet count in adult and paediatric patients were different for each aberrant antigen. The

Table 2. Frequency of individual antigen aberrancy in Acute Lymphoblastic Leukaemia

Category	CD marker	Frequency
Aberrant expression of myeloid markers on Acute Lymphoblastic Leukaemia (n=149)	CD13	8 (5.3%)
	CD33	5 (3.3%)
	CD15	5 (3.3%)
	CD14	2 (1.3%)
	CD117	2 (1.3%)
	MPO	1 (0.6%)
Aberrant expression of T-cell markers on B-cell Acute Lymphoblastic Leukaemia (n=133)	sCD3	3 (2.2%)
	cCD3	3 (2.2%)
	CD4	2 (1.5%)
	CD7	14 (10.5%)
	CD56	3 (2.2%)
Aberrant expression of B-cell markers on T-cell Acute Lymphoblastic Leukaemia (n=16)	CD79a	2 (12.5%)

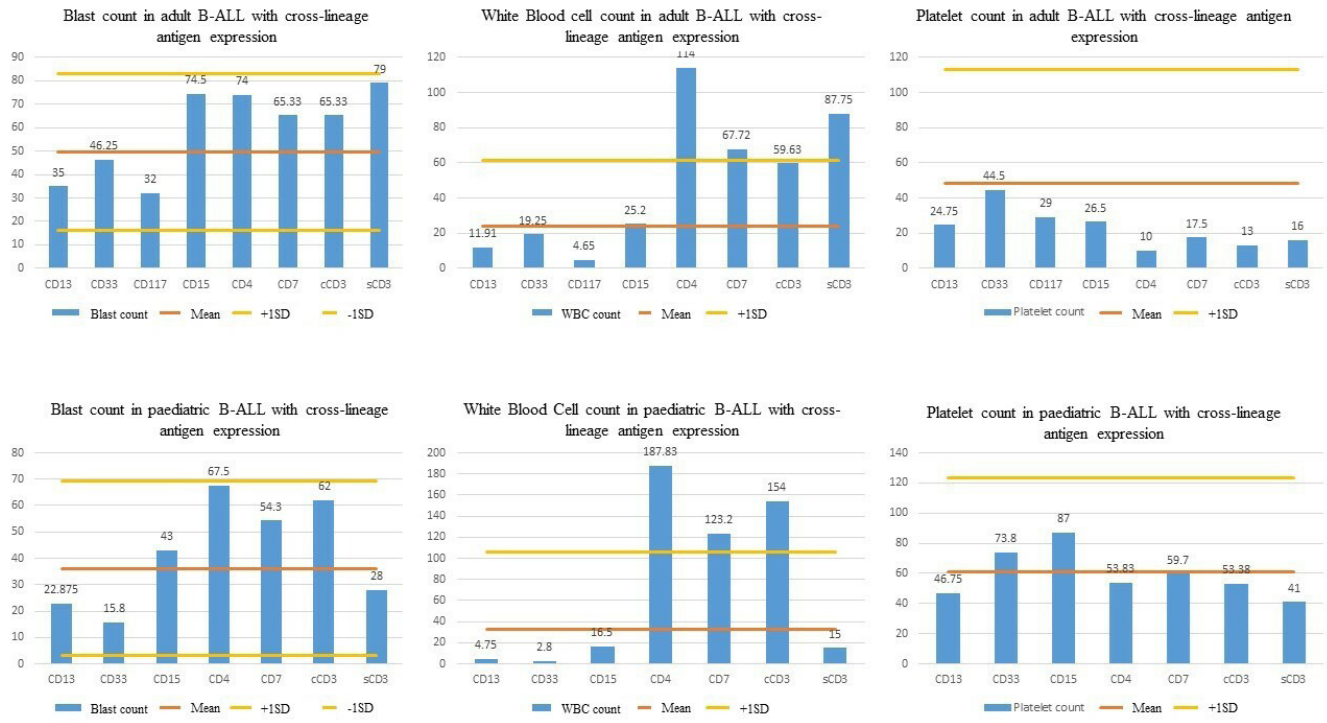


Figure 3: Blast count, white blood cell count, and platelet count at diagnosis in B-cell Acute Lymphoblastic Leukaemia with cross-lineage antigen expression in adult and paediatric patients.

mean ±SD for complete blood count parameters, at diagnosis, concerning individual aberrant antigens, in adult and paediatric B-ALL is shown in **Figure 3**. Some patients showed clinical features which were associated with specific antigen aberrancies. A summary of associations of statistical significance between individual antigenic aberrancy and blast count, platelet count, and clinical features is shown in **Table 3**.

Table 3. Statistically significant associations between individual cross-lineage antigen expression and blast count, platelet count and clinical features

Cross-lineage aberrant antigen in B-cell Acute Lymphoblastic Leukaemia		Blast count (p-value)	Platelet count (p-value)	Presentation (p-value)
Adult	CD117 (n=2)	↓(0.027)	--	Weakness (0.041), Gum hypertrophy (0.038)
	CD15 (n=5)	↑(0.027)	--	--
	CD14 (n=2)	↓(0.027)	↓(0.010)	--
	CD7 (n=4)	--	--	Cough (0.04)
	cCD3 (n=3)	--	--	Cough (0.02)
	sCD3 (n=2)	--	--	Mediastinal mass (0.038)
Paediatric	CD56 (n=3)	↑(0.001)	--	Anaemia (0.014)
	CD7 (n=10)	--	--	Lymphadenopathy (0.04)
	sCD3 (n=1)	↑(0.001)	↓(0.014)	--

↑- Higher than the mean count
↓- Lower than the mean count
-- - No significant associations

3.4 Relationship of cross lineage antigen expression with complete blood count parameters in the induction phase and during the maintenance phase

Myeloid antigen expression showed lower than mean blast count and white blood cell count, and a higher than mean platelet count in the induction phase. Blasts were not seen in the maintenance phase in any of these cases. Although these associations were not statistically significant, the findings were clinically relevant. The relation of myeloid antigen expression with white blood cell count and platelet count is shown in **Figure 4**.

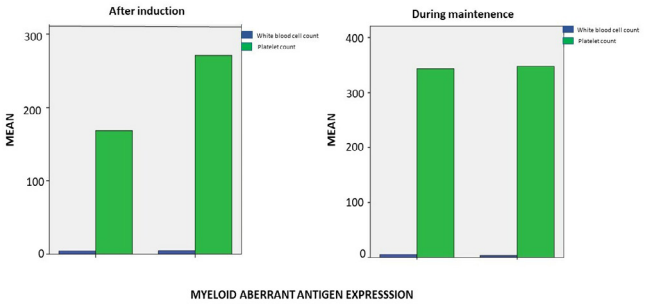


Figure 4: Association of aberrant myeloid antigen expression with white blood cell count and platelet count in the post-induction phase and during the maintenance phase.

4. Discussion:

Diagnosis and prognosis of ALL are based on clinical features, morphological examination, immunophenotype, molecular genetics, and post-induction minimal residual disease status [8,18]. In this study, 149 cases of ALL were included. The male-to-female ratio was similar to as reported in previous studies [8,11,18]. Thirty (20.1%) cases showed aberrant expression of at least one cross-lineage antigen. This frequency varied widely from 9.8 to 70.3% among different studies [15,19,20]. Commonly expressed aberrant myeloid markers in ALL are CD13, CD33, CD117, and CD15. Their reported frequency in ALL varies widely among different studies. Supriyadi et al in their review found that expression of myeloid antigens on ALL ranged between five to 36%, and their study reported 25% of ALL cases to show aberrance of myeloid antigens [8]. Lopes et al reported a higher frequency of 49.2% among paediatric ALL.6 Indian studies, mainly representing the population from Northern India showed 42.5% and 64.7% of all ALL cases to have aberrant myeloid antigen expression. [16,20,21] We found a lower frequency in this study. Only 11 (7.3%) of all cases of ALL showed myeloid antigen expression. Most studies have reported CD13 and CD33 as the commonest myeloid aberrant antigens expressed in ALL [11, 15, 21]. Putti et al suggested that the co-expression of CD13 and CD33 on ALL is so

common that it may be considered a representative of all myeloid antigens seen in ALL.[12] Aberrant CD13 expression ranges from 15.6 – 32.3%.[13, 17] Aberrant CD33 expression ranges from 14.3 – 22.8% [11,13]. In the present study, CD13 was the most common myeloid antigen, and was seen in five (3.3%) patients. CD33 expression was also seen in only five (3.3%) patients.

The significance of aberrant expression of myeloid markers in ALL remains controversial. World Health Organisation 2008/2016 has recognized the frequent association of CD13 aberrance with B-ALL with ETV6-RUNX1 translocation, which has a good prognosis [14]. In contrast, some studies have noted the expression of myeloid markers in ALL in relation to poor response to therapy and prognosis.[11,23] A few studies have reported expression of CD13 is associated with lower than median complete blood count parameter values [24,25]. Sharma et al reported that there was a statistically significant decrease in white blood cell count and blast count in ALL patients with myeloid antigens [16]. Contrary to this, a few studies have reported no association of aberrant myeloid antigen expression with initial white blood cell count [10, 13]. Lopes et al noted a lower than median platelet count in children lacking myeloid markers in ALL thus increasing the risk of bleeding in the absence of myeloid aberrance [6]. Differing from this, Bhushan et al and Supriyadi et al reported that myeloid antigen expression in patients with ALL had no association with clinical/biological features, response to therapy, or long-term outcome [8, 25,26]. Hence, no consensus has been reached yet. In this study, we found that expression of either CD13 or CD33 was associated with lower blast count, lower white blood cell count, and a lower platelet count at diagnosis, this feature is clinically relevant, though not statistically significant.

The aberrant expression of CD117 in ALL varies between 10.3 – 54% [11,27,28]. Sarma et al reported CD117 to be the most commonly expressed myeloid antigen in their study [26]. World Health Organisation 2008/2016, however, considers the aberrant expression of CD117 on ALL to be rare [13, 14]. CD117 may be used as a surrogate for Feline McDonough Sarcoma-like tyrosine kinase 3 (FMS-like FLT3) mutation and patients benefit from FLT3 inhibitors [28].

In this study, we found CD117 expression in only two (1.3%) patients, and it was associated with lower than mean blast count, white blood cell count, and platelet count. The reduction in blast count was statistically significant (p=0.027). In addition, this study showed adult patients with CD117 expression to have a significant association with weakness (p=0.041) and gum hypertrophy (p=0.038) at presentation. Previous studies, however, found no association between the aberrancy of CD117 with clinical presentation [13, 25].

Aberrant expression of CD15 in ALL is rare and has been found only in a few studies [13,15,29]. CD15 expression in ALL is associated with the KMT2A-rearranged type of B-ALL, which is associated with poor prognosis [14,30] CD15 is linked to a greater risk of relapse, regardless of other prognostic factors. [31]. We found aberrance of CD15 was associated with higher than mean blast count and this was found to be statistically significant (p=0.027). White blood cell count and platelet count were lower than the mean value. However, it had no statistical significance.

Rare expression of myeloperoxidase was observed in a few studies. Suggs et al and Mazher et al reported two and one cases respectively.[10, 18] We had a single case of paediatric B-ALL that was dim positive for myeloperoxidase.

The expression of T-cell markers in B-ALL is uncommon and ranges between 7 – 13.4% [17,31,32]. In this study, among 133 cases of B-ALL, the most commonly expressed aberrant T-cell marker was CD7, seen in 14 (10.5%) cases. This was followed by CD4, which was seen in only two (1.5%) cases. Previous studies have, however, reported CD4 to be the most common T-cell aberrancy on B-ALL, followed by CD7 [17, 20, 32]. In this study, patients with aberrant expression of CD7 had higher than mean blast count, white blood cell count, and lower than mean platelet count in adult and paediatric B-ALL however, this had no statistical significance. It was noted that patients with CD7 aberrancy had cough and mediastinal mass at presentation. These clinical features had significant statistical correlation (p=0.04).

A few studies have reported aberrant expression of CD3 on B-ALL. Shahni et al reported 3% of B-ALL cases to express aberrant surface CD3 [20]. This was similar to our observation of the expression of cytoplasmic CD3 (dim) and surface CD3 (variable) in three (2.2%) patients each. Patients, in this study, with aberrant surface CD3 expression had a significant association with cough (p=0.02) and mediastinal mass (p=0.038) at presentation. Expression of surface CD3

blast count but this association was not of statistical significance. Cytoplasmic CD3 expression did not show any specific relation with clinical presentation or changes in blood counts.

The presence of aberrant expression of the Natural-Killer (NK) cell marker, CD56, was observed in a few studies and its frequency ranges from 3.1 – 5.1% [30, 32]. According to World Health Organisation 2016, this expression pattern is not associated with any specific cytogenetic abnormality. In this study, three (2.2%) cases of B-ALL expressed CD56. Patients with CD56 expression had higher than mean blast count and this was statistically significant (p=0.001).

Aberrant B-cell marker expression on T-ALL has been reported to be 15% [17]. In this study, out of 16 cases of T-ALL, two (12.5%) cases showed B cell antigen expression. Both cases expressed CD79a. The frequency of CD79a expression on T-ALL is reported to range between 16 – 47.3% [17, 28]. Aberrant cytoplasmic CD79a expression did not have any statistically significant associations with complete blood count parameters or clinical presentation.

The opinion relating the expression of aberrant antigens and response to treatment is varied. Literature supports the presence of myeloid antigen aberrancy on lymphoblasts as a key factor in poor response to treatment and delayed remission [10, 12]. However, there was no difference in the survival of patients with T-ALL with and without expression of myeloid antigens [10]. In contrast to this, studies have also shown no association of cross-lineage antigen expression with response to treatment, concluding that these expression profiles should not be used for any therapeutic choices [13].

In this study, patients with cross-lineage myeloid antigen expression had lower than mean blast count in the post-induction phase. No blasts were seen during the maintenance phase. However, there was no significant association between any cross-lineage aberrancy with post-induction phase and maintenance phase counts.

Limitations of the study: Due to retrospective design, the number of cases in the study is less to arrive at a definitive conclusion about the effect of cross lineage antigen expression on prognosis. Event-free survival and overall survival were not assessed in this study due to a lack of long-term follow-up. Minimal residual disease assays were outsourced and hence the disease status was not accessible in all cases. A larger prospective analysis of treatment naïve ALL for cross-lineage antigen expression and their effect on blood counts at predefined intervals (during induction, post-induction, and maintenance phase) along with long-term follow-up to assess their association with prognosis is likely to overcome the limitations.

5. Conclusion

The frequencies of cross-lineage antigen expression are different in various populations across the globe. Myeloid antigen expression is common in ALL. However, these have no significant prognostic implications. Aberrant CD13 and CD33 expressions are common in adult B-ALL; and, CD7 and CD3 in paediatric B-ALL. Aberrant expression of CD117, CD15, CD14, CD56, and CD3 have a significant association with the blast count, white blood cell count, and platelet count at diagnosis. The presence of cross-lineage antigen expression influences the blast count, white blood cell count, and platelet count in the induction phase and maintenance phase. This finding is clinically relevant though not statistically significant.

Acknowledgment

The authors would like to acknowledge and thank the flow cytometry technical staff of Kasturba Hospital laboratory, MAHE, Manipal, for their contribution toward this study.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1- Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. Br J Haematol. 2011 May;153(4):421-36. doi: 10.1111/j.1365-2141.2011.08595.x. https://pubmed.ncbi.nlm.nih.gov/21385170/

2- Peters JM, Ansari MQ. Multiparameter flow cytometry in the diagnosis and management of acute leukaemia. Arch Pathol Lab Med. 2011 Jan;135(1):44-54. doi: 10.5858/2010-0387-RAR.1. PMID: 21204710. https://pubmed.ncbi.nlm.nih.gov/21204710/

3- Jalal SD, Al-Allawi NAS, Al Doski AAS. Immunophenotypic aberrancies in acute lymphoblastic leukemia from 282 Iraqi patients. Int J Lab Hematol. 2017 Dec;39(6):625-632. doi: 10.1111/ijlh.12716. Epub 2017 Jul 18. PMID: 28722319. https://pubmed.ncbi.nlm.nih.gov/28722319/

4- Cherian S, Hedley BD, Keeney M. Common flow cytometry pitfalls in diagnostic hematopathology. Cytometry B Clin Cytom. 2019 Nov;96(6):449-463. DOI: 10.1002/cyto.b.21854. Epub 2019 Nov 7. PMID: 31697047. https://pubmed.ncbi.nlm.nih.gov/31697047/

5- Bagg A. Lineage ambiguity, infidelity, and promiscuity in immunophenotypically complex acute leukemias: genetic and morphologic correlates. Am J Clin Pathol. 2007 Oct;128(4):545-8. doi: 10.1309/NQQQY37HPDNAMC1Y. PMID: 17875503. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.939.2484&rep=rep1&type=pdf

6- Lopes TC, Andrade KN, Camelo NL, et al. Influence of aberrant myeloid expression on acute lymphoblastic leukemia in children and adolescents from Maranhão, Brazil. Genet Mol Res. 2014 Dec 4;13(4):10301-7. DOI: 10.4238/2014.December.4.25. PMID: 25501242. https://pubmed.ncbi.nlm.nih.gov/25501242/

7- Liu J, Tan X, Ma YY, et al. Study on the Prognostic Value of Aberrant Antigen in Patients With Acute B Lymphocytic Leukaemia. Clin Lymphoma Myeloma Leuk. 2019;19:349-58. doi:10.1016/j.clml.2019.03.012. https://pubmed.ncbi.nlm.nih.gov/31076372/

8- Supriyadi E, Veerman AJ, Sutaryo, et al. Myeloid Antigen Expression in Childhood Acute Lymphoblastic Leukemia and Its Relevance for Clinical Outcome in Indonesian ALL-2006 Protocol. J Oncol. 2012;2012:135186. doi: 10.1155/2012/135186. Epub 2012 Nov 26. PMID: 23227046; PMCID: PMC3513752. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3513752/

9- Al-Zaabi M, Al-Khabori M, Fawaz N, et al. Impact of Aberrant Myeloid Antigen Expression on Outcomes of Patients with T-cell Acute Lymphoblastic Leukemia. Oman Med J. 2017 May;32(3):189-193. doi: 10.5001/omj.2017.36. PMID: 28584598; PMCID: PMC5447802. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5447802/

10- Suggs JL, Cruse JM, Lewis RE. Aberrant myeloid marker expression in precursor B-cell and T-cell leukemias. Exp Mol Pathol. 2007 Dec;83(3):471-3. DOI: 10.1016/j.yexmp.2007.08.012. Epub 2007 Sep 7. PMID: 17963747. https://pubmed.ncbi.nlm.nih.gov/17963747/

11- Sobol-Milejska G, Mizia-Malarz A, Wos H. Expression of myeloid antigens on lymphoblast surface in childhood acute lymphoblastic leukemia at diagnosis and its effect on early response to treatment: a preliminary report. Int J Hematol. 2013 Sep;98(3):331-6. DOI: 10.1007/s12185-013-1397-6. Epub 2013 Jul 24. PMID: 23881645. https://pubmed.ncbi.nlm.nih.gov/23881645/

12- Putti MC, Rondelli R, Cocito MG, et al. Expression of myeloid markers lacks prognostic impact in children treated for acute lymphoblastic leukemia: Italian experience in AIEOP-ALL 88-91 studies. Blood. 1998 Aug 1;92(3):795-801. PMID: 9680347. https://pubmed.ncbi.nlm.nih.gov/9680347/

13- Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th Ed. Lyon: International Agency for Research on Cancer; c2017.

14- Abdulateef NA, Ismail MM, Aljedani H. Clinical significance of co-expression of aberrant antigens in acute leukemia: a retrospective cohort study in Makah Al Mukaramah, Saudi Arabia. Asian Pac J Cancer Prev. 2014;15(1):221-7. DOI: 10.7314/apjcp.2014.15.1.221. PMID: 24528030. https://pubmed.ncbi.nlm.nih.gov/24528030/

15-Zhang YD, Tan LN, Hu Q, et al. Immunophenotyping and its clinical significance in childhood acute lymphoblastic leukemia. Zhongguo Dang Dai Er Ke Za Zhi. 2012 Mar;14(3):188-91. PMID: 22433405. https://pubmed.ncbi.nlm.nih.gov/22433405

16- Sharma M, Sachdeva MU, Varma N, et al. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: A study from Northern India. J Cancer Res Ther. 2016 Apr-Jun;12(2):620-6. DOI: 10.4103/0973-1482.147716. PMID: 27461621. https://pubmed.ncbi.nlm.nih.gov/27461621/

17- Cézar RS, Cerqueira BA, da Paz Sde S, et al. Outcome of B-Cell Acute Lymphoblastic Leukemia in Brazilian Children: Immunophenotypical, Hematological, and Clinical Evaluation. J Pediatr Hematol Oncol. 2015 Aug;37(6):423-8. DOI: 10.1097/MPH.0000000000000358. PMID: 26056790. https://pubmed.ncbi.nlm.nih.gov/26056790/

18- Mazher N, Malik N, Imran A, et al. Aberrant Expression of CD Markers in Acute Leukaemia. Ann. Pak. Inst. Med. Sci. 2013;9:99-102. https://apims.net/apims_old/Volumes/Vol9- 2/ABERRANT%20EXPRESSION%20OF%20CD%20MARKERS%20IN%20ACUTE%20LEUKEMIA.pdf

19- Amirghofran Z, Daneshbod Y, Gholijani N, et al. The Influence of Bcl-2 and Myeloid Antigen Expression on Response to Therapy in Childhood Acute Lymphoblastic Leukaemia. Archives of Iranian Medicine. 2011;14:170. magiran.com/p862976. https://pubmed.ncbi.nlm.nih.gov/21529104/

20- Shahni A, Saud M, Siddiqui S, et al. Expression of aberrant antigens in hematological malignancies: A single center experience. Pak J Med Sci. 2018 Mar-Apr;34(2):457-462. doi: 10.12669/pjms.342.13996. PMID: 29805426; PMCID: PMC5954397. https://pubmed.ncbi.nlm.nih.gov/29805426/

21- Sharma RK, Purohit A, Somasundaram V, et al. Aberrant myeloid antigen co-expression is correlated with high percentages of CD34-positive cells among blasts of acute lymphoblastic leukaemia patients: an Indian tertiary care center perspective. Blood Res. 2014;49(4):241–5. doi:10.5045/br.2014.49.4.241. https://www.bloodresearch.or.kr/journal/view.html?uid=1779&vmd=Full

22- Dalal BI, Al Mugairi A, Pi S, et al. Aberrant expression of CD13 identifies a subgroup of standard-risk adult acute lymphoblastic leukaemia with inferior survival. Clin Lymphoma Myeloma Leuk. 2014;14:239–44. doi:10.1016/j.clml.2013.10.003. https://www.sciencedirect.com/science/article/abs/pii/S2152265013004618

23- Vitale A, Guarini A, Ariola C, et al. Absence of prognostic impact of CD13 and/or CD33 antigen expression in adult acute lymphoblastic leukemia. Results of the GIMEMA ALL 0496 trial. Haematologica. 2007 Mar;92(3):342-8. doi: 10.3324/haematol.10385. PMID: 17339183. https://pubmed.ncbi.nlm.nih.gov/17339183/

24- Ibrahim AM, Hameed BM. Prognostic value of myeloid antigens expression in childhood acute lymphoblastic leukaemia. Iraqi J Haematol. 2017 May;6:12-6. doi:10.4103/ijh.ijh_5_17. https://www.ijhonline.org/article.asp?issn=2072-8069;year=2017;volume=6;issue=1;spage=12;epage=16;aulast=Ibrahim

25- Bhushan B, Chauhan PS, Saluja S, et al. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. Clin Exp Med. 2010 Mar;10(1):33-40. DOI: 10.1007/s10238-009-0067-8. Epub 2009 Sep 25. PMID: 19779962. https://pubmed.ncbi.nlm.nih.gov/19779962/

26- Sarma A, Hazarika M, Das D, et al. Expression of aberrant CD markers in acute leukemia: a study of 100 cases with immunophenotyping by multiparameter flowcytometry. Cancer Biomark. 2015;15(4):501-5. DOI: 10.3233/CBM-150482. PMID: 25835176. https://pubmed.ncbi.nlm.nih.gov/25835176/

27- Garg N, Kotru M, Kumar D, et al. Correlation of expression of aberrant immunophenotypic markers in T-ALL with its morphology: A pilot study. J Lab Physicians. 2018 Oct-Dec;10(4):410-413. DOI: 10.4103/JLP.JLP_35_18. PMID: 30498313; PMCID: PMC6210842.a. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6210842/
28- Rodríguez-Rodríguez S, Pomerantz A, Demichelis-Gómez R, et al.e Impact of Aberrant Antigens in the Outcome of Patients with Acute Leukaemia at a Referral Institution in Mexico City. Rev Invest Clin. 2016;68:305-13. https://pubmed.ncbi.nlm.nih.gov/28134942/
29- Seegmiller AC, Kroft SH, Karandikar NJ, et al. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. Am J Clin Pathol. 2009 Dec;132(6):940-9. DOI: 10.1309/AJCP8G5RMTWUEMUU. PMID: 19926587. https://pubmed.ncbi.nlm.nih.gov/19926587/
30- Lenkei R, Björk O, Ost A, et al. Expression of CD15 as predictor of relapse in children with acute lymphoblastic leukaemia of the pre-B type. Leuk Res. 1991;15:189–94. doi:10.1016/0145-2126(91)90120-i. https://www.sciencedirect.com/science/article/abs/pii/014521269190120I
31- Hussein S, Gill KZ, Sireci AN, et al. Aberrant T-cell antigen expression in B lymphoblastic leukaemia. Br J Haematol. 2011 Nov;155(4):449-56. doi: 10.1111/j.1365-2141.2011.08870.x. Epub 2011 Sep 19. PMID: 21923643. https://pubmed.ncbi.nlm.nih.gov/21923643/
32- Paietta E, Neuberg D, Richards S, et al. Rare adult acute lymphocytic leukemia with CD56 expression in the ECOG experience shows unexpected phenotypic and genotypic heterogeneity. Am J Hematol. 2001 Mar;66(3):189-96. DOI: 10.1002/1096-8652(200103)66:3<189::aid-ajh1043>3.0.co;2-a. PMID: 11279625. https://pubmed.ncbi.nlm.nih.gov/11279625/