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Aberrant Antigen Expression in Acute Lymphoblastic Leukemia and Its Correlation with Post-Induction Remission

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Abstract

Objective: To determine the aberrant expression of antigens in newly diagnosed patients of Acute Lymphoblastic Leukemia and its correlation with post induction remission

Methodology: This Descriptive cross-sectional study was carried out at department of Hematology, Armed Forces Institute of Pathology (AFIP), from April 2024 to August 2025. A total of 183 newly diagnosed patients with ALL of all ages and both genders were enrolled. Morphology, cytochemistry and flow cytometric immunophenotyping were used to analyze bone marrow and peripheral blood samples to identify lineage and aberrant antigen expression. Induction chemotherapy was given to patients over a period of 29 days and the remission was assessed by bone marrow biopsy. Analysis of the data was done by SPSS 22.0, Chi-square test was used; a p-value of ≤ 0.05 was taken as significant.

Results: Aberrant antigen expression was identified in 36 (19.7%) of 183 ALL patients (median age 13 years; 69.9% male). B-ALL and T-ALL comprised 152 (83.1%) and 31 (16.9%) cases respectively. CD33 and CD13 were most frequently expressed aberrant antigens. The total number of patients who achieved remission were 149 (81.4%), however, it was significantly less among patients who had aberrant antigen expression (24 (66.7%) vs. 125 (85.0%), p = 0.011). Persistent thrombocytopenia, increased residual blasts and persistently raised WBC counts were also linked to aberrant antigen expression.

Conclusions: Aberrant antigen expression in ALL was associated with sub-optimal treatment response and poor post-induction outcome, highlighting its significance as a prognostic variable in the therapeutic decision-making.

Keywords: Acute Lymphoblastic Leukemia, Aberrant antigen expression, Immunophenotyping, Remission, Flow Cytometry.

Introduction

Acute Lymphoblastic Leukemia (ALL) is one of the most common cancers and accounts for the most common leukemia in the pediatric population. While presence of lymphoblasts is the diagnostic morphological finding, yet patients have variable clinical presentations and response to treatment which can be explained by distinct immunophenotypic findings, cytogenetic features and presence or absence of molecular genetic markers.[1] Thus, the diagnosis of Acute Lymphoblastic leukemia (ALL) is made by integrating morphology and cytochemistry with immunophenotyping and underlying molecular genetic features.[2]

Immunophenotyping by flow cytometry is a modality that is used for diagnosis of acute leukemia and monitoring of minimal residual disease (MRD). Immunophenotyping is used to identify CD markers (Cluster of differentiation antigens) which are cellular antigens expressed by clonal blast population in ALL.[3] Immunophenotyping not only establishes the lineage but also sub-characterizes patients of Acute Lymphoblastic Leukemia. CD3 is a lineage specific marker of T-cells and CD19 is a lineage specific marker for B-cells. Common cell surface markers expressed by B cells include CD19, CD20, CD22 and CD79a, while T cells express CD3, CD5, CD4, CD8 and CD7 on their surface.[4,5]

Clonal cells of Acute Lymphoblastic leukemia occasionally express CD markers which remain unexplained in the normal cellular differentiation pathways of their lineage. Detection of these aberrant CD markers on clonal cells is referred to as “leukemia-associated aberrant immunophenotype” or “aberrant antigen expression” [6]. Myeloid associated CD markers are most common type of aberrant antigens expressed by cells in B-ALL and sometimes in T-ALL. These markers include CD13, CD33, CD15 and CD117. [7]

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Flow cytometric immunophenotyping plays a vital role in detecting aberrant antigen expression. This not only helps in characterizing these patients but has important prognostic implications. Expression of these aberrant antigens determines response to treatment and clinical course.[8,9]

Most of the research on aberrant expression and its prognostic impact has been done in western countries. There is no comprehensive study done in the Pakistani population on aberrant antigen expression in ALL and determining its role in response to treatment. The aim of this study is to determine the immunophenotypic pattern and aberrant expression of antigens in newly diagnosed patients of ALL and to study its correlation with post induction remission. The results of this study will help us to compare response of patients having aberrant antigen expression to induction chemotherapy with those not having aberrant antigen expression and to assess any association of aberrant antigen expression with remission status in our population.

Methodology

This descriptive cross-sectional study was carried out at Department of Hematology, Armed Forces Institute of Pathology for duration of 8 months after the approval of synopsis by CPSP and ethical approval by the Institutional Review Board of the AFIP (IRB/24/3117, approved on 10 June 2024). The sample size came out to be 180 using WHO calculator by keeping confidence level of 95% and absolute precision of 4%. Anticipated population having aberrant antigen expression in ALL came out to be 8.03% from previous study which showed expression of aberrant markers in B-ALL to be around 4.6% and in T-ALL to be around 3.43% [10]. Non probability consecutive sampling was carried out.

Inclusion criteria: All new patients of Acute Lymphoblastic Leukemia were enrolled in the study after verbal and written informed consent. Patients of all ages and both genders were included.

Exclusion criteria: Patients already on chemotherapy and those who failed to continue chemotherapy due to any reason were excluded. Relapsed cases and patient lost to follow up were also excluded.

Bone marrow biopsy of all patients was performed at AFIP after informed consent and under aseptic conditions. Peripheral blood or bone marrow sample for immunophenotyping were collected in EDTA sampling tube at the time of bone marrow biopsy. Immunophenotyping by flowcytometry was carried out at Armed Forces Institute of Pathology to identify lineage of blasts and presence or absence of aberrant antigen expression. Acute Lymphoblastic Leukemia was diagnosed according to World Health Organization (2022) Criteria on the basis of morphological assessment of peripheral blood and bone marrow biopsy and immunophenotyping [11]. Patients were given induction chemotherapy for 29 days. Post induction bone marrow examination was carried out on 29th day of Induction chemotherapy to assess remission status. Treatment response was compared between two groups, one comprising of patients having aberrant antigen expression and other comprising of those not having aberrant antigen expression.

Complete blood counts were performed on seven-part automated hematology analyzer Sysmex XN-3000. Peripheral blood smears and bone marrow aspirate slides were stained with Leishman stain and Giemsa stain. Sudan black B and Acid phosphatase were applied in cytochemistry. Trephine biopsy was processed and stained with hematoxylin and eosin stain (H&E). Immunophenotyping by flowcytometry was carried out on Becton Dickinson Fluorescence-Activated Cell Sorter. Antibody panel used for flowcytometry included Cyto CD3, Cyto CD79a, CD5, CD10, CD19, CD13, CD33, CD117, HLADR, CD45, CD14, MPO, CD34 and CD5. When required, antibody panel was extended to include CD4, CD2, CD8 and Tdt.

Data was analyzed by using Statistical Package for Social Sciences (SPSS) 22.00. Normality of data was checked by Shapiro-Wilk test that showed that age was non-normally distributed and represented by using median (IQR). Qualitative data was represented by using percentage and frequency. Chi square test (for qualitative variables), was applied and Relative risk with 95% confidence interval (CI) calculated. The p-value of ≤ 0.05 was considered as statistically significant.

Results

Out of 183 patients, 128 (69.9%) patients were male and 55 (30.1%) patients were females. Median age was 13.00 (27.00 – 3.00) years ranging from 1 to 60 years. Among

clinical features, fever was the most common symptom (54.1%), followed by easy fatigability (16.4%), bruising (11.5%), bleeding (10.9%), pallor (4.4%), and abdominal pain (2.7%). Lymphadenopathy was observed in 27.3% of patients. Organomegaly was present in 43.7% of cases, with splenomegaly in 17.5%, hepatomegaly in 8.7%, and hepatosplenomegaly in 17.5% cases. Aberrant antigen expression was noted in 36 (19.7%) patients as shown in Table-I.

Table-I: Baseline Characteristics of Patients (n = 183)

Variables		n (%)
Gender	Male	128 (69.9%)
	Female	55 (30.1%)
Aberrant expression	Yes	36 (19.7%)
	No	147 (80.3%)
Age (years)	Median (IQR)	13.00 (27.00 – 3.00)
Symptoms	Fever	99 (54.1%)
	Bleeding	20 (10.9%)
	Bruises	21 (11.5%)
	Easy fatiguability	30 (16.4%)
	Palour	8 (4.4%)
	Abdominal Pain	5 (2.7%)
Lymphadenopathy	Present	50 (27.3%)
	Absent	133 (72.7%)
Organomegaly	Splenomegaly	32 (17.5%)
	Hepatomegaly	16 (8.7%)
	Hepatosplenomegaly	32 (17.5%)

Out of 183 cases, 152 were B-ALL and 31 were of T-ALL. Aberrant antigen expression was seen in 24 cases (15.7%) of B-ALL and 12 cases (38.7%) of T-ALL. CD33 was most frequently expressed aberrant CD marker in B-ALL followed by CD13 and CD117. Among T-ALL patients, CD117 was most frequently expressed aberrant CD marker present in 6 (19.3%) out of 31 cases. There were three cases of T-ALL which showed expression of aberrant CD marker of B lineage i.e. CD79a as shown in table II.

Amongst the B-ALL markers, CD19 (82.5%) and CD79a (81.4%) were the most frequently expressed and CD10 was positive in 74.3% of cases. Among T-ALL markers, CD3 was detected in 16.9% of 183 cases, followed by CD5 in 13.7%, CD2 in 7.7%, and both CD4 and CD8 in 6.0% each. CD34 was positive in (78.1%), HLA-DR (88.5%) and Terminal deoxynucleotidyl transferase (Tdt) was observed in 19.1% of patients. Among aberrant antigen markers, CD33 was most frequently expressed CD marker in ALL (including B and T-ALL) which was positive in 10.4% of cases, CD13 in 7.7%, and CD117 in 4.4% as shown in Table-III.

Table-III: Frequency of all CD Markers in ALL cases (n=183)

Markers	Frequency (%)
B-ALL Markers	
CD 10	136 (74.3%)
CD 19	151 (82.5%)
CD 79a	149 (81.4%)
T-ALL Markers	
CD 3	31 (16.9%)
CD 4	11 (6.0%)
CD 8	11 (6.0%)
CD5	25 (13.7%)
CD2	17 (7.7%)
Markers of immaturity	
CD34	143 (78.1%)
Tdt	35 (19.1%)
HLADR	162 (88.5%)
Aberrant Expression Markers	
CD13	14 (7.7%)
CD33	19 (10.4%)
CD117	8 (4.4%)
CD79a in T-ALL	3 (1.6%)

Patients with aberrant antigen expression had a lower remission rate compared to those without aberrant antigen expression (24 (66.7%) vs 125 (85.0%), p=0.011). The relative risk (RR) was 0.352 (95% CI: 0.154–0.806), indicating that aberrant antigen expression was associated with a reduced likelihood of achieving remission. A statistically significant association was

Table-II: Frequency of Aberrant CD markers in B-ALL and T-ALL

Type of leukemia	Total no. of cases (n=183)	Cases with aberrant antigen expression	Aberrant CD markers	Frequency of CD markers
B -ALL	152 (83.1%)	24 (15.7%)	CD33	10 (6.5%)
			CD13	4 (2.6%)
			CD117	2 (1.3%)
			CD13+CD33	8 (5.2%)
T-ALL	31(16.9%)	12 (38.7%)	CD33	1 (3.2%)
			CD13	2 (6.4%)
			CD117	6 (19.3%)
			CD79a	3 (9.6%)

observed between aberrant antigen expression and increased blast percentage (p = 0.011). Patients with aberrant antigen expression were more likely to have blasts ≥5%, with an RR of 0.352 (95% CI: 0.154–0.806), indicating that aberrant antigen expression was associated with more probability of having increased blast percentage after treatment. For platelet counts before treatment, there was no significant association with aberrant antigen expression (p = 0.812). The relative risk (RR) was 0.903 (95% CI: 0.387–2.104), suggesting comparable rates of thrombocytopenia between those with and without aberrant antigen expression. However, for platelet counts after treatment, a highly significant association was observed (p < 0.001) i.e. patients with aberrant antigen expression were more likely to have low platelet counts, with an RR of 6.850 (95% CI: 2.663–17.618), indicating a strong link between aberrant antigen expression and persistent thrombocytopenia post treatment. For hemoglobin levels, no significant association was found either before treatment (p = 0.730, RR = 1.176, 95% CI: 0.468–2.952) or after treatment (p = 0.500, RR = 1.370, 95% CI: 0.547–3.426). For WBC count, significant association was found both before treatment (p = 0.042, RR = 0.432, 95% CI: 0.190 – 0.938) and after treatment (p = 0.016, RR = 0.380, 95% CI: 0.171 – 0.847). This suggests that aberrant antigen expression do significantly influence WBC counts. The association between aberrant antigen expression and lymphadenopathy was not statistically significant (p = 0.534) (Table-IV).

Table-IV: Association of Aberrant antigen expression with clinical and laboratory parameters

Variables	Aberrant antigen expression		p-value*	RR (95% CI)
	Present (n=36)	Absent (n=147)		
Remission status				
In remission	24 (66.7%)	125 (85.0%)	0.011	0.352 (0.154 – 0.806)
Not in remission	12 (33.3%)	22 (15.0%)		
Blast count				
< 5 %	24 (66.7%)	125 (85.0%)	0.011	0.352 (0.154 – 0.806)
≥ 5 %	12 (33.3%)	22 (15.0%)		
PLT (×10⁹/L) at diagnosis				
Low	27 (75.0%)	113 (76.9%)	0.812	0.903 (0.387 – 2.104)
Normal	09 (25.0%)	34 (23.1%)		
Hb at diagnosis				
Low	27 (79.4%)	105 (76.6%)	0.730	1.176 (0.468 – 2.952)
Normal	7 (20.6%)	32 (23.4%)		
WBC count at diagnosis				
Normal	9 (25.0%)	64 (43.5%)	0.042	0.432 (0.190 – 0.938)
High	27 (75.0%)	83 (56.5%)		
Post induction PLT (×10⁹/L)				
Low	12 (33.3%)	10 (6.8%)	< 0.001	6.850 (2.663 – 17.618)
Normal	24 (66.7%)	137 (93.2%)		
Post induction Hb				
Low	18 (69.2%)	69 (62.2%)	0.500	1.370 (0.547 – 3.426)
Normal	8 (30.8%)	42 (37.8%)		
Post induction WBC count				
Normal	23 (63.9%)	121 (82.3%)	0.016	0.380 (0.171 – 0.847)
High	13 (36.1%)	26 (17.7%)		
Lymphadenopathy				
Present	8 (22.2%)	42 (28.6%)	0.534	0.714 (0.301– 1.694)
Absent	28 (77.8%)	105 (71.4%)		

Discussion

Aberrant antigen expression was found in 36 patients (19.7%), most commonly myeloid markers (CD33 and CD13) were seen in this cohort of 183 newly diagnosed ALL patients. More importantly, aberrant expression was linked to a much lower post-induction complete remission rate and with relatively poor hematological recovery mostly persistent thrombocytopenia and increased residual marrow/peripheral blasts. These findings represent that aberrant immunophenotypes in our population are associated with adverse treatment response. We identified aberrant antigen expression in 36 (19.7%) cases of ALL, most commonly CD33 in 19 (10.4%) and CD13 in 14 (7.7%) cases. This is in agreement with a reported Pakistani cohort with 17.8% prevalence (CD33 12.3%, CD13 11%)[12] and an Egyptian series with 40% prevalence (CD33 23.3%, CD13 16.7%). Although there are variations in the overall frequency, the

general similarity in predominance of CD33 and CD13 in the studies supports the generalizability of our results.[13]

Induction remission in the aberrant expression patients (24 (66.7%) vs 125 (85.0%), p=0.011) was significantly lower and this is consistent with other studies, which have associated myeloid or cross-lineage antigen expression with poor initial response and delayed marrow recovery. Venugipalan et al., 2023 stated that aberrant immunophenotypes related to leukemia were related to worse early hematologic recovery and increased residual disease at follow-up.[14] Sivakumar and Basu, 2021 also found that delayed platelet recovery post induction was correlated with aberrant immunophenotypes, which supports the notion that these indicators negatively influence marrow recovery.[15] Conversely, other larger studies, including the GIMEMA ALL-0496 trial did not show any significant effect of CD13/CD33 expression on remission or survival.[16] Similarly, in a real-life cohort of 1005 adult B-ALL patients, Liao et al., 2023 showed that aberrant CD13/CD33 expression was associated with increased minimal residual disease (MRD) positivity but did not significantly decrease remission rates.[17]

Aberrant expression was associated with increased post-induction blast percentages and a substantially increased risk of persistent thrombocytopenia (RR = 6.85) in our dataset, which demonstrates delayed hematological recovery. This can be a sign of biological resistance of aberrant clones or delayed recovery of marrow after therapy. Similar signs of delayed recovery and a larger residual disease with myeloid antigen positivity have also been observed in institutional cohorts, which also contributes to the adverse prognostic importance of aberrant expression.[18] It is significant that we found aberrant expression to be associated with unfavorable WBC dynamics, but not hemoglobin or lymphadenopathy. A study by Mansoor et al. also showed discordance between clinical features and immunophenotypic aberrancies, which may indicate that their role is mostly limited to the kinetics of treatment but not the presentation at baseline.[19] Recent research based on the use of modern flow cytometry and molecular profiling has shown that aberrant antigen expression is biologically distinct leukemic subclones with different therapy responses. These subclones are defined by changes in the sensitivity to treatment, and this is the reason why aberrant expression is associated with lower remission rates, persistent thrombocytopenia, and increased residual blast burden found in our patients. These results demonstrate the prognostic importance of aberrant immunophenotypes and support their role in risk stratification models and decision-making.[11,20]

In general, our findings contribute to the accumulating body of evidence that aberrant antigen expression is a negative prognostic factor in ALL. The same pattern of decreased remission and limited recovery of marrow in our study and in multiple studies around the region underscores the importance of recording aberrant markers at baseline and to factor them into the risk stratification model. Multicenter studies that incorporate both molecular profiling and MRD analysis should be considered in future to further establish their prognostic and treatment implication in our population.

Conclusion

Aberrant antigen expression was found in close to one-fifth of patients with Acute Lymphoblastic leukemia and was strongly linked to reduced remission rates, increased residual blasts, and poor hematological recovery. These results confirm that aberrant immunophenotypes, in this case, CD33 and CD13, are unfavorable prognostic factors in our population and indicate the significance of including detailed immunophenotyping in early risk stratification and treatment planning.

LIMITATION: This study had limited generalizability, inadequate power to conduct subgroup analysis, no MRD evaluation, no cytogenetic and molecular correlation, and could not assess long-term outcomes, including relapse and survival. These limitations underscore the importance of larger and longitudinal and genetically combined studies.

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