Research Article

Prognostic effect of aberrant flowcytometric markers on outcome of children with acute lymphoblastic leukemia (Oncology Center - Mansoura University experience)

Menna M. Zedan[1], Naness A. Salem[2], Suzy Abd ELMabood[1], Metwaly I. Mortada[3], Bothina M. Hasaneen[4]

[1] Pediatric Hematology, Oncology and Bone Marrow Transplant Unit, Department of Pediatrics, Mansoura University Children's Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2] Pediatric Endocrinology Unit, Department of Pediatrics, Mansoura University Children's Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[3] Hematology Unit, Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

[4] Pediatric Neurology Unit, Department of Pediatrics, Mansoura University Children's Hospital, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

Corresponding Author: Assistant lecturer of Pediatrics, Mansoura University Children's Hospital, Mansoura Faculty of Medicine, Mansoura, Egypt. Email: dr.menna.zidan.ped@gmail.com. ORCID: 0000-0001-9561-4379.Assistant lecturer of Pediatrics, hematology, oncology, and bone marrow transplant unit, Mansoura University Children's Hospital, Mansoura Faculty of Medicine,Mansoura, Egypt. Email: dr.menna.zidan.ped@gmail.com. ORCID: 0000-0001-9561-4379.

Professor of Pediatrics/endocrinology, endocrine Unit, Mansoura University Children's Hospital, Mansoura Faculty of Medicine, Mansoura, Egyp
Email: nanees.salem@gmail.com. ORCID: 0000-0001-6783-9095.
Professor of Pediatrics/hematology, oncology, and bone marrow transplant unit, Mansoura University Children's Hospital, Mansoura Faculty of Medicine, Mansoura, Egypt
Email: suzyabdelmabood@yahoo.com. ORCID: 0000-0002-2241-2589.
Assistant professor of clinical pathology, hematology unit, Mansoura Faculty of Medicine, Mansoura, Egypt
Email: metwalymortada@gmail.com. ORCID: 0000-0003-3465-1955
Professor of Pediatrics / Neurology, Neurology Unit, Mansoura University Children's Hospital, Mansoura Faculty of Medicine, Mansoura, Egypt.
Email: bothinammh@mans.edu.eg. ORCID: 0000-0002-0279-5527

Abstract

Objective: While the available literatures provide conflicting evidence regarding both the prevalence and prognostic significance of aberrant antigen expression in children with B-acute lymphoblastic leukemia (B-ALL). This study aimed to determine its frequency and clinical impact, mainly on disease outcome.

Methods: We reviewed the records of children less than 18 years at diagnosis of B- ALL. The electronic data files were retrieved from Jan 2011 to December 2021. Only patients categorized as standard risk group were included in our study. The patients were followed for at least till the end of their treatment protocol.

Results: Out of 159 cases, 53 (33.3%) exhibited expression of aberrant lineage antigens. Among all aberrantly expressed myeloid antigens, CD33 was the most common, present in 24 (15.1%) patients, followed by CD13 in 19 patients (11.9%). Relapse was significantly higher among the positive aberrant lineage group compared to the negative group (43.40% Vs 18.90%, respectively, p =0.001**).** After multivariate adjustment, aberrant antigen lineage expression remained an independent determinant of reduced disease-free survival (DSF) (HR = 3.37). When evaluating individual antigen clusters, CD33 positivity was identified as a specific independent predictor, carrying a 2.28-fold higher hazard for reduced DFS (HR = 2.28).

Conclusion: In this cohort, CD33 and CD13 are the most frequent aberrant markers; importantly, CD33 expression serves as a significant independent predictor for relapse. CD33 expression may identify a higher-risk subgroup that could benefit from closer monitoring and risk-adapted therapeutic strategies to improve long-term outcomes in children with B-ALL.

Keywords: B-acute lymphoblastic leukemia (B-ALL)- CD33- CD13 – aberrant lineage antigen expression - disease-free survival (DFS)-relapse.

1. Introduction

Acute lymphoblastic leukemia (ALL) arises from uncontrolled proliferation of lymphoblasts, resulting in a marked reduction of functional hematopoietic cells within the bone marrow [1]. Within the pediatric population, it remains the predominant cancer, comprising approximately one-quarter of all pediatric cancers and the majority of childhood leukemias, with peak incidence observed between two and five years of age [2].

The prognosis of ALL depends on several patient-specific and disease-related characteristics. The Pediatric Oncology Group (POG) and Children's Cancer Group (CCG) established a common set of risk stratification criteria [3].

The introduction of risk-adapted, response-based chemotherapy protocols has markedly improved survival rates for pediatric ALL, with global outcomes now exceeding 85%. Despite these advances, approximately 10–15% of children

experience relapse irrespective of treatment intensity, rendering relapsed ALL as one of the principal causes of cancer-associated death among children [4].

Diagnosis of leukemia had been advanced after the introduction of Flow cytometric immunophenotyping, which plays a pivotal role in delineating leukemic lineage characteristics and detecting aberrant antigen expression [5].

In B-cell acute lymphoblastic leukemia (B-ALL), leukemic blasts consistently express B-lineage markers such as CD19, CD79a, and CD22, detectable by flow cytometry. These cells also show immature phenotypic features, including TdT, CD34, and dim CD45 [6].

Leukemias that exhibit antigens from more than one hematopoietic lineage without fulfilling criteria for bi-phenotypic or bilineal leukemia are described as having aberrant antigen expression [7].

In B- acute lymphoblastic leukemia (B-ALL), "The most consistently reported aberrancies were those of myeloid antigens, with CD13 and CD33 predominating. Aberrant T lymphoid marker like CD3, CD5 and CD7 have also been reported [6]

The positivity threshold for these aberrant markers is typically more than 20% of leukemic cells [8]. Many studies suggested that aberrant antigen expression carries prognostic significance. and may negatively impact clinical response, remission rate, and diminish overall survival in leukemic patients [9, 10].

The clinical significance and prognostic implications of aberrant antigen expression remain controversial [11]. The present study is designed to evaluate the prognostic relevance of aberrant lineage antigen expression among children with B-ALL. Recognizing these atypical immunophenotypic patterns is crucial for accumulating reliable data on their frequency and understanding their clinical and prognostic implications, helping oncologists to optimize therapeutic strategies.

2. Patients and methods

2.1 Study design

A retrospective cohort design was employed.

2.2 Patients

Our present study included **159** children patients with B-ALL and treated at the Oncology Center of Mansoura University in the period from January 2011 to December 2021. The study was conducted in compliance with the principles of the Declaration of Helsinki and received approval from the Mansoura Faculty of Medicine Institutional Research Board (MD.23.07.792.R1.R1).

Included patients fulfilled standard risk criteria defined as age between one and less than ten years at presentation, initial total leucyocytic count <50,000/mm^3, and absence of extramedullary disease [3]. Also included a study group that completed their chemotherapy treatment protocol at the Oncology Center of Mansoura University.

Patients who met initially high-risk criteria (e.g., age <1 year or ≥10 years at diagnosis, initial WBC ≥50,000/mm^3, non-B immunophenotypes such as T ALL or presence of extramedullary disease) [3], patients not attaining full morphological remission at day 29 following induction therapy, or those who lost follow-up were excluded.

2.3 Methodology

2.3.1 ALL diagnosis

ALL diagnosis was established primarily through bone marrow evaluation, requiring ≥25% blast cells, and was further validated by flow cytometric studies. [12].

2.3.2 Clinical and laboratory data retrieval

All clinical data, including date and age at diagnosis, gender, presence of extramedullary disease (such as testicular or CNS involvement), treatment outcome (complete remission, refractory disease, or relapse), as well as the type and timing of relapse, were retrieved from electronic records. In addition, Laboratory data, including complete blood

counts, results of peripheral blood film, bone marrow aspiration, and CSF results assessed at diagnosis, after induction chemotherapy, and at relapse, and flow cytometric analysis.

2.3.3 Treatment protocols

The management of ALL was based on the modified Berlin-Frankfurt-Münster (BFM)- 90 regimen [13].

2.3.4 Definition and classification of relapse

An M3 marrow exhibiting over 25% blasts after the achievement of complete remission was considered indicative of bone marrow relapse, in the absence of concurrent CNS or testicular affection. Isolated CNS relapse was diagnosed either by the presence of blasts in cerebrospinal fluid with a leukocyte count ≥5/µL, or by clinical and radiologic evidence of central nervous system affection. Diagnosis of isolated testicular relapse required histologic confirmation by biopsy. Combined relapse referred to CNS and/or testicular involvement occurring together with marrow relapse, defined as either M2 (≥5% blasts) or M3 infiltration [14]. Most studies define relapse occurring within the first 18 months from diagnosis as early relapse, between 18 and 36 months as intermediate, and beyond 36 months as late relapse [14].

2.3.5 Immunophenotyping

Immunophenotyping reports were reviewed with emphasis on leukemia-associated profiles and antigen expression patterns. Before therapy, multiparametric flow cytometry was carried out on samples from peripheral blood and/or bone marrow.

The Navios EX Flow Cytometer was utilized for data acquisition and analysis under established laboratory protocols. Instrument calibration and optimization followed established procedures, with application settings adjusted according to manufacturer specifications. Marker staining was performed according to validated protocols, and the cell concentration was adjusted to approximately 1×10^6 cells per 100 µL before antibody incubation. Four primary tubes were processed, each stained with an eight-color antibody panel.

Monoclonal antibodies were conjugated to fluorochromes including FITC, APC, APC-H7, PE, PE-Cy7, PerCP-Cy5.5, V450, and V500. All reagents were obtained from Becton Dickinson Biosciences, USA. B-Lymphoid lineage was identified using CD10, CD19, CD20, CD22, and CD79a. Aberrant myeloid expression was assessed through CD33, CD13, and MPO, while monocytic markers included CD4, CD11b, CD11c, CD14, CD36, and CD64. In addition, aberrant T-cell lineage-associated markers such as CD1a, CD2, CD3, CD5, CD7, and CD58. Immaturity was further defined by CD34, HLA-DR, CD117, and TdT [15]

Antigen expression was interpreted according to predefined thresholds. Positivity for a surface marker was defined as antigen expression in over 20% of the gated cell population, whereas cytoplasmic markers were considered positive if reactivity was observed in over 10% of events. [16].

2.4 Statistical analysis:

The SPSS software (version 24, IBM Corp, Armonk, NY, USA) was used for data analysis. Mean and Standard Deviation (SD) or median and interquartile range (IQR) were used to express quantitative data, while numbers (N) and percentages were used to express qualitative data (%). For quantitative variables, parametric data comparison was performed using the Independent Student's t-test and non-parametric data was performed using the Mann-Whitney U test. "The Chi-Square test was employed to compare qualitative variables, while Fisher's exact test was used in instances where over 20% of cells had expected counts less than five.

Survival outcomes, including DFS and Overall Survival (OS), were estimated using the Kaplan–Meier method, with the Log-rank test utilized to determine the statistical significance of differences between curves. Independent prognostic factors were identified using univariate and multivariate Cox proportional hazards regression models with Hazard Ratios (HR) and 95% confidence intervals (CI) calculated. Statistical significance was defined as a p-value below 0.05.

3. Results

3.1 Patient characteristics and the impact of aberrant antigen expression on clinicopathological characteristics

A total of 159 children diagnosed with standard-risk B-ALL disease were included in this study. The mean age at initial diagnosis was 5.80 ± 2.57 years. The sex distribution was nearly equal. Baseline hematological parameters were reported in Table 1. Regarding immunophenotypic subtypes, the common B-ALL subtype predominated. Circulating peripheral blast was expressed in 40.0 % of the cases. Aberrant lineage antigen expression was identified in 33.3% of the cohort (Table 1).

The two groups, the aberrant-positive patients (n = 53) and the aberrant-negative patients (n = 106), showed comparable sex and age of dignosis. Also, no significant differences in hematological parameters at initial presentation were observed, and blast percentages, likewise, did not differ either at diagnosis or at relapse. A significantly higher proportion of patients in the aberrant lineage–positive group presented with the Pro-B subtype (Table 2).

Patients with aberrant antigen positivity experienced relapse considerably more often than those without (43.4% vs. 18.9%). Overall survival was lower among patients with aberrant lineage antigen expression (64.0% vs. 84.0%), with no significant variation in age or timing of death. Long-term follow-up demonstrated that aberrant-positive patients had a lower survival rate (p = 0.008), while death attributable to relapse was higher in this group (p .34.0% vs. 16.0%) (Table 3).

3.2 Immunophenotypic analysis

Aberrant lineage antigen expression profiling showed CD33 positivity in 24 patients (15.1 %), CD13 in 19 (11.9%), CD117 in 7 (4.4%), CD3 and CD5 each in 4 (2.5%), CD2 in 3 (1.9%), CD7 and CD4 each in 2 (1.3%), CD11 in 2 (1.3%), CD119 in 1 (0.6%), and CD8 in 1 (0.6%). Some aberrant markers were co-expressed, most notably CD33/CD13. (Figure 1)

3.3 Impact of aberrant lineage antigen expression on OS and DFS

Overall survival analysis (OS) between both groups initially showed nearly comparable outcomes, with one-year survival reaching 98.1% in each. Differences began to emerge by three years, as survival in the aberrant-positive group dropped to 77.4% compared with 88.7% in the aberrant-negative group. The difference further increased at five years (67.0% vs. 86.5%) and was sustained at ten years, with long-term survival maintained in the aberrant-negative group (81.6%) but reduced to 57.1% among aberrant-positive patients. (Figure 2).

Disease-free survival (DFS) analysis initially showed comparable outcomes at one year (96.2% vs. 94.3%). However, by three years, survival in the aberrant-positive group had declined to 75.3% compared to 84.9% in the aberrant-negative group. This difference increased further at five years (58.6% vs. 81.7%) and persisted at ten years, with long-term survival maintained in the aberrant- negative group (80.2%) but reduced to 52.7% among the aberrant-positive group (Figure 3).

3.4 Cox regression for DFS

In univariate analysis using Cox regression for factors affecting DFS, the Pro-B subtype, aberrant lineage antigen expression, age at initial diagnosis, and level of hemoglobin were all significantly linked with reduced DFS. Moreover, after the multivariate analysis, aberrant lineage antigen expression remained a significant independent predictor (HR = 3.37). In addition, both age at initial diagnosis and hemoglobin level remain statistically significant, indicating that older age and higher hemoglobin at presentation were linked to a modestly increased hazard of relapse. On the contrary, sex, platelet count, and total leukocyte count were not significant predictors. (Table 4).

Further analysis for DFS adjusted for aberrant lineage clusters confirmed the adverse impact of immunophenotypic markers. In univariate analysis, the Pro-B subtype, CD13 positivity, CD33 positivity, age at diagnosis, and hemoglobin level were all significantly associated with lower DFS.

In the multivariate analysis, CD33 was a significant relapse predictor (HR = 2.28, p = 0.05), suggesting a potential independent effect. Furthermore, age at diagnosis (HR = 1.10, 95% CI: 1.01–1.20, p = 0.04) and hemoglobin level (HR = 1.26, 95% CI: 1.07–1.47, p = 0.01) were independent predictors, indicating that older age and higher

hemoglobin at presentation modestly increased relapse risk. Together, these results confirm the sustained adverse influence of aberrant lineage antigen expression, with CD33 positivity serving as a potentially significant prognostic marker.

Table 1. Demographic, hematological, flow-cytometric, and immunophenotypic data of the included patients

	Total participants (n= 159)
Current age (years) Mean ± SD	13.2 ± 4.88
Age at diagnosis (years) Mean ± SD	5.80 ± 2.57
Sex No. (%)	
Male	81 (50.9%)
Female	78 (49.1%)
Haemoglobin (g/dL) Mean ± SD	7.57 ± 2.18
Platelet count (×10³/µL) Median (IQRs)	34 (18, 61)
Total leukocytic count (×10³/µL) Median (IQRs)	9 (4, 16)
Absolute neutrophil count (×10³/µL) Median (IQRs)	0.7 (0.29, 1.70)
Lactate dehydrogenase (U/L) Median (IQRs)	500 (319, 820)
Peripheral blood blasts N (%)	
Yes	62 (40.0%)
No	97 (60.0%)
Bone marrow blasts at diagnosis (%) Mean ± SD	86.8 ± 11.8
Bone marrow blasts at induction chemotherapy (%) Mean ± SD	1.79 ± 0.82
Bone marrow blasts at relapse (%) Mean ± SD	63.4 ± 21.8
Treatment outcome N (%)	
Complete remission	115(73.0%)
Relapse	43 (27.0%)
B-subtype	
Common B	139 (87.3%)
Pro-B	20 (12.6%)
Aberrant lineage expression	
Yes	53 (33.3%)
No	106 (66.7%)

SD: standard deviation; IQR: interquartile range

Table 2. Demographic, hematological, blast cell indices, and immunophenotypic characteristics of study groups

	Aberrant lineage		
	Positive (n= 53)	**Negative (n= 106)**	**p value**
Current age (years) Mean ± SD	12.50 ± 4.62	13.48 ± 4.97	0.32[1]
Age at diagnosis (years) Mean ± SD	5.72 ± 3.47	5.84 ± 3.64	0.84[1]
Sex No. (%)			0.31[2]
Male	30 (56.60%)	51 (48.10%)	
Female	23 (43.40%)	55 (51.90%)	
Hemoglobin (g/dL) Mean ± SD	7.14 ± 1.57	7.79 ± 2.42	0.08[1]
Platelet count (×10³/µL) Median (IQRs)	32 (18, 52)	36 (21, 64.80)	0.51[3]
Total leukocytic count (×10³/µL) Median (IQRs)	8.20 (4, 16)	9.75 (4, 17)	0.89[3]
Absolute neutrophil count (×10³/µL) Median (IQRs)	0.60 (0.30, 1.58)	0.70 (0.24, 2)	0.65[3]
Bone marrow blasts at diagnosis (%) Mean ± SD	88.08 ± 9.78	86.11 ± 12.67	0.33 [1]
Bone marrow blasts after induction chemotherapy (%) Mean ± SD	1.81 ± 0.97	1.78 ± 0.90	0.87 [1]
Bone marrow blasts at relapse (%) Mean ± SD	61.80 ± 32.58	66.71 ± 32.15	0.74 [1]
B-subtype			
Common B	39 (73.60%)	100 (94.30%)	***<0.001*[2]**
Pro-B	14 (26.40%)	6 (5.70%)	

SD: standard deviation; IQR: interquartile range; [1] Independent Student's t-test [2] Chi square test, [3] Mann–Whitney U test. significant P value <0.05.

Table 3. Relapse characteristics and survival outcomes in both study groups

	Aberrant lineage		
	Positive group (n= 53)	**Negative** (n= 106)	**p value**
Relapse	23 (43.40%)	20 (18.90%)	***0.001***[2]
Timing of relapse			
Early	6 (26.10%)	6 (30.0%)	
Intermediate	7 (30.40%)	9 (45.0%)	0.42[2]
Late	10 (43.50%)	5 (25.0%)	
Time to relapse (years) Median (IQRs)	1.92 (1.33, 3.75)	1.88 (1.48, 2.56)	0.42[3]
Age at relapse (years) Mean ± SD	9.04 ± 4.50	9.14 ± 4.20	0.94[1]
Type of relapse			
Medullary	10 (43.50%)	6 (30.00%)	
CNS	4 (17.40%)	9 (45.00%)	
CNS + medullary	8 (34.80%)	4 (20.00%)	0.19 [2]
Ocular	0 (0.00%)	1 (5.00%)	
Testicular + medullary	1 (4.30%)	0 (0.00%)	
Overall survival status			
Survivors	34 (64.20%)	89 (84.0%)	***0.005*** [2]
Non-survivors	19 (35.80%)	17 (16.0%)	
Age at death (years) Mean ± SD	9.21 ± 4.37	10.50 ± 4.12	0.37 [1]
Time to death (years) Median (IQRs)	2.00 (1.58, 3.29)	2.50 (1.92, 3.75)	0.64 [3]
Survival outcome of relapse patients			
Survivors	5 (21.70%)	3 (15%)	0.57 [2]
Non-survivors	18 (78.30%)	17 (85%)	
Final outcome			
On follow-up	29 (54.70%)	85 (80.20%)	
Death by relapse	18 (34.0%)	17 (16.0%)	
Relapse (alive)	5 (9.40%)	3 (2.80%)	***0.008***[2]
Death without relapse	1 (1.90%)	0 (0.00%)	
Transplanted	0 (0.00%)	1 (0.90%)	

SD: standard deviation; IQR: interquartile range; [1] Independent Student's t-test [2] Chi square test, [3] Mann–Whitney U test. significant P value <0.05.

Table 4. Univariate and multivariate Cox Regression Analysis for Disease- Free Survival among the Included Patients Adjusted for Aberrant Lineage Presence

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Subtype (Pro B vs Common B)	2.24	1.06–4.72	***0.03***	1.64	0.72–3.76	0.24
Aberrant line	2.77	1.47–5.22	***0.002***	3.37	1.59–7.14	***0.001***
Age at diagnosis	1.08	0.99–1.17	0.07	1.10	1.01–1.21	***0.03***
Haemoglobin	1.17	1.02–1.35	0.03	1.32	1.11–1.57	***0.002***
Platelets	1.00	1.00–1.01	0.59	1.00	1.00–1.00	0.91
Total leucocytic count	1.02	0.99–1.04	0.24	1.02	0.99–1.04	0.16

HR: Hazard ratio, CI: confidence interval, test: Cox Regression analysis, significant P value <0.05.

Table 5. Univariate and Multivariate Cox Regression Analysis for Disease- Free Survival among the Included Patients adjusted for aberrant lineage clusters (n= 159)

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Sex (Male vs Female)	1.31	0.69–2.46	0.41	1.11	0.57–2.16	0.77
Subtype (Pro B vs Common B)	2.24	1.06–4.72	***0.03***	1.52	0.58–3.99	0.40
CD13	2.18	1.04–4.60	***0.04***	1.88	0.72–4.88	0.19
CD33	2.55	1.27–5.14	***0.01***	2.28	1.00–5.22	***0.05***
CD117	2.41	0.74–7.87	0.15	3.69	0.94–14.43	0.06
CD3	0.80	0.11–5.86	0.83	1.12	0.14–8.66	0.92
CD5	1.80	0.43–7.49	0.42	1.61	0.35–7.28	0.54
Age at diagnosis	1.08	0.99–1.17	0.07	1.10	1.01–1.20	***0.04***
Haemoglobin	1.17	1.02–1.35	***0.03***	1.26	1.07–1.47	***0.01***
Platelets	1.00	1.00–1.01	0.59	1.00	1.00–1.00	0.89
Total leucocytic count	1.02	0.99–1.04	0.24	1.02	1.00–1.05	0.11

HR: Hazard ratio, CI: confidence interval, test: Cox Regression analysis, significant P value <0.05.

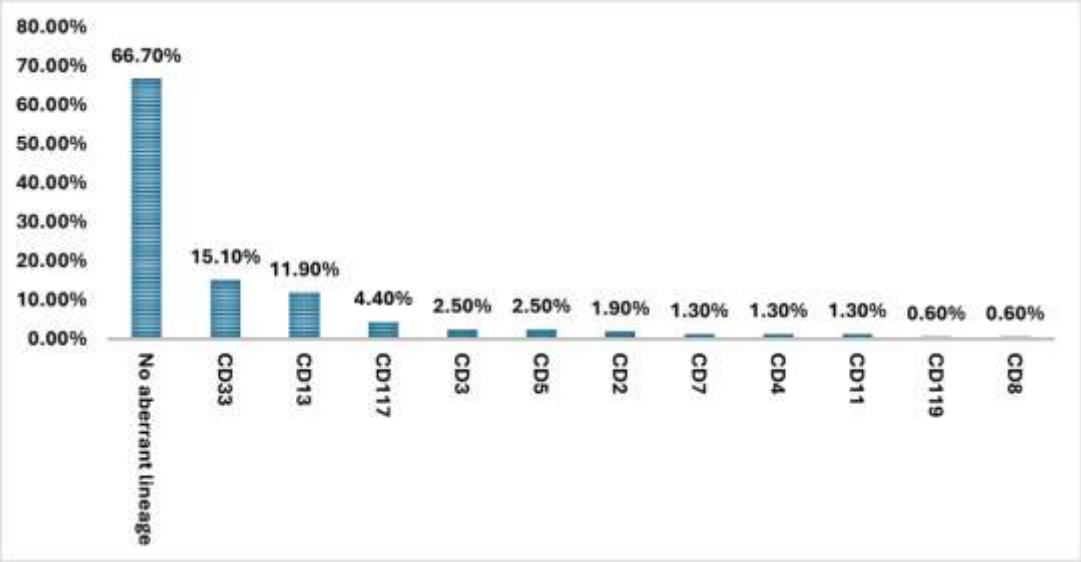

Fig. 1 Displayed the Frequency of Aberrant Antigen Expression in Patients with B-ALL. The most frequent ones in B-ALL are CD33 (15.1%) followed by CD13 (11.9%). NB: total frequency of aberrant markers exceeds the number of aberrant positive cases because of antigen-co-expression

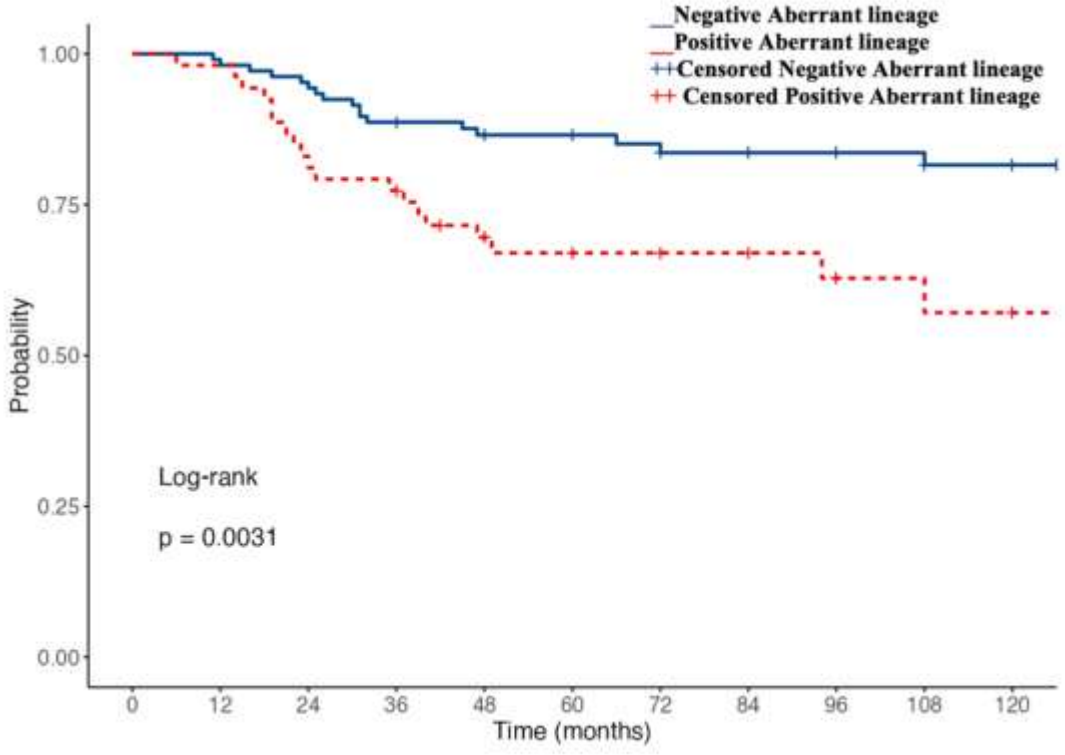

Fig. 2 Kaplan- Meier curve analysis for OS demonstrating significantly better overall survival among patients with negative aberrant lineage (Log- Rank *p< 0.0031*)

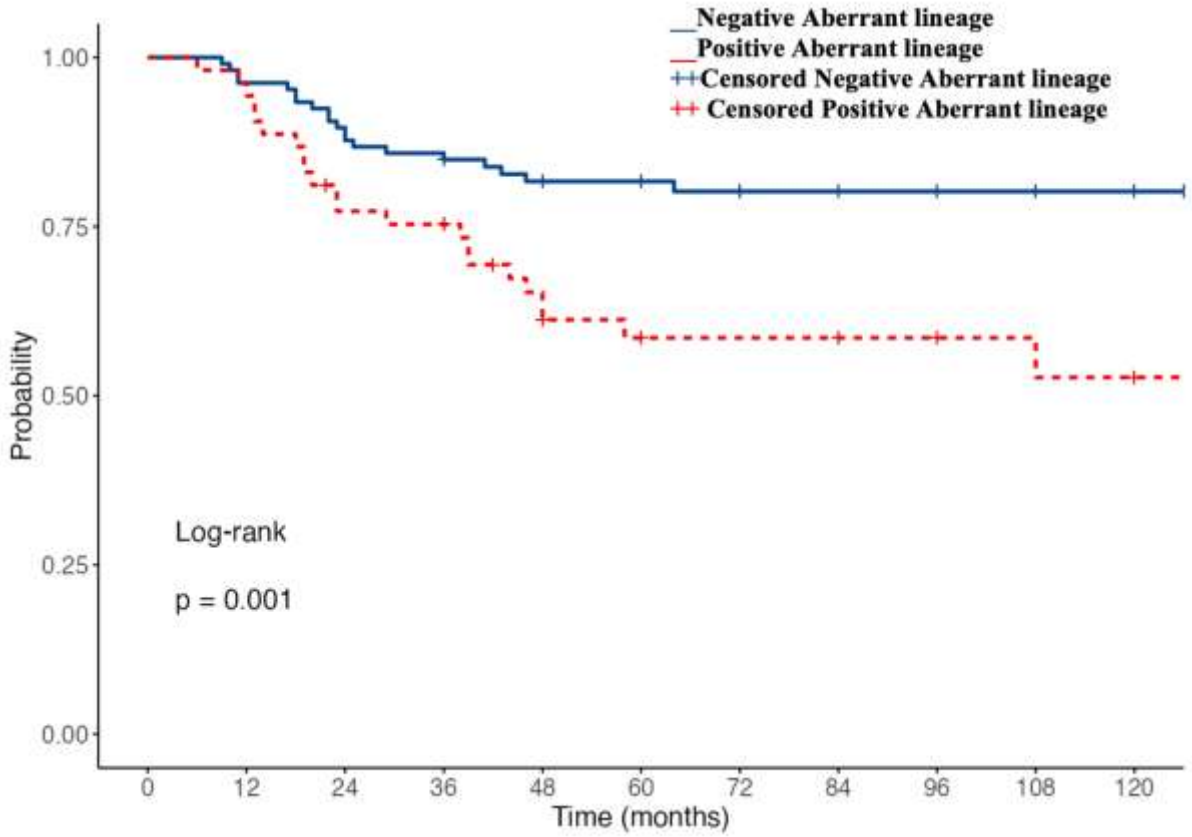

Fig. 3 Kaplan- Meier curve analysis for DFS demonstrated significantly better disease- free survival among patients with negative aberrant lineage (Log- Rank *pp< 0.001*)

4. Discussion

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, marked by the uncontrolled replication of lymphoblasts within the bone marrow. While flow cytometric immunophenotyping is the cornerstone for current diagnostic standards for determining lineage, the emergence of aberrant lineage expression has become a topic of growing clinical interest. The objective of this work was to determine whether aberrant antigen expression carries prognostic significance in children diagnosed with B-ALL for better designing a risk-based management plan. In this study, 159 pediatric patients were enrolled with an average age at initial diagnosis of 5.80 years. This age distribution is consistent with the global epidemiological peak for pediatric (B-ALL) reported in previous studies [17, 18].

The male-to-female ratio was nearly equal, which is consistent with previous studies reporting no sex predilection among children with B- ALL [19, 20]. 40 % of cases showed blast on peripheral circulation, whereas 60 % showed no circulating blasts, necessitating bone marrow aspiration for definitive diagnosis, irrespective of peripheral findings [21]. Additionally, our study demonstrated a clear predominance of the common B-ALL subtype. This aligns with established epidemiological patterns of (B -ALL), where the common B-ALL subtype consistently represents the majority of cases [22].

Among our study, 53 patients of a total of 159 had aberrant antigen expression, representing 33,3%. CD33 was the most prevalent aberrant antigen, expressed in 45% of aberrant cases, followed by CD13 in 35%. This frequency is highly consistent with approximately 30.67% reported by Sivakumar et al. [22] and Rodríguez-Rodríguez et al [23, 24].

The aberrancy rate in our study is lower than that identified in Iraq by Al-Badran et al., who reported a rate of 44.8%, and Sharma et al., who found a rate of 42.5% [25, 26].

The distribution frequency of aberrant markers highlights the predominance of myeloid antigens, particularly CD33 and then CD13, as the most common aberrant markers in our study, while T-lineage antigens were relatively rare. Consistent with our result, Sivakumar et al. identified CD33 as the prominent myeloid antigen in B-ALL, with CD13 and CD15 occurring less frequently, while T-lineage markers such as CD2 and CD7/CD8 were less common [23]. Similarly, in the study by Mostafa et al., CD33 was identified as the leading aberrant myeloid marker, followed by CD13, alongside CD7 [27].

In contrary with our findings, Saqlain et al. documented that in B-ALL patients, among the aberrant markers, CD13 was identified most frequently, followed by CD5, CD2, and CD117[28].

The divergence in these frequencies may be attributed to differences in the studies methdology, participants' demographics, variability of the immunophenotyping panels used, differences in flow cytometry instruments, the use of fresh vs. frozen material, and the specific reagent clones used [19, 28].

No age disparity at diagnosis was observed between the aberrant positive and negative groups, which is consistent with other cohorts [9, 26].

Furthermore, hematological parameters, peripheral blast, and bone marrow blast, whether on initial diagnosis or relapse, did not vary significantly between the ALE-positive and ALE-negative groups in our study. This absence of correlation is validated by many studies [23, 25].

Markedly divergent from other reports. Khaled et al. noted that CD13 expression was linked with a significantly higher WBC count [29]. Similarly, Moustafa et al. reported that platelet count was lower in aberrant B-ALL patients [27]. On the contrary, Lopes et al. reported that patients lacking myeloid antigen expression had notably lower platelet count [10].

These opposite findings reflect differences in the patient demographics and the specific aberrant markers analyzed. Overall, it seems that the link between hematological parameters and aberrant antigen expression is variable and non-consistent.

There was a higher prevalence of the Pro-B subtype among aberrant lineage antigen-positive patients, indicating that cross-lineage antigen expression may be linked with less-differentiated B-cell progenitors. Despite the strong association identified in our study, several reports had no significant correlation between B-cell maturation stage and aberrant antigen expression [10, 25]

Regarding outcome, aberrant positive patients showed significantly inferior outcomes compared with aberrant-negative cases. Overall survival was markedly reduced with more than a two-fold increase in mortality and higher relapse-related deaths. While one-year DFS was comparable between groups, a pronounced divergence evolved over time, with five and ten-year DFS declining to 58.6% and 52.7% in the aberrant-positive group versus 81.7% and 80.2% in aberrant-negative patients. The same for Overall survival curves, confirming the adverse prognostic impact of aberrant antigen expression, which was strongly associated with increased relapse risk and consequently a diminished long-term survival.

On concordance with our result, Khaled et al. reported markedly shorter survival for B-ALL patients with CD33 expression [29]. Mendoza et al. reported that patients expressing myeloid markers B-lineage lymphoblasts exhibited a lower OS [7]. Similarly, Bhushan et al. highlighted that CD33 aberrancy was linked with adverse clinical outcomes [30].

This association can be traced back to reports from the 1990s, when several groups first highlighted the prognostic relevance of phenomena of aberrant antigen expression in children with B -ALL and its link with lower OS [31, 32].

Unlike the adverse survival outcomes documented in our study and others, Supriyadi et al. reported that myeloid antigen positivity was not prognostically relevant for DFS or OS [19]. Moreover, findings from the United Kingdom's Medical Research Council indicated that CD13 and CD33 expression carried no prognostic significance [33].

Differences in treatment intensity may explain Outcome variability. The adverse prognostic influence of myeloid antigen presence in B-ALL appears to diminish as therapeutic efficacy improves, particularly in Western protocols where event-free survival often reaches 80–90%.

A differential glucocorticoid sensitivity is another explanation; myeloid-negative blasts are more susceptible to steroid-induced cytotoxicity, whereas myeloid-positive blasts show relative resistance, likely due to intrinsic drug-resistance mechanisms. This reduced steroid sensitivity may contribute to poorer outcomes in aberrant-positive patients[19].

Rodríguez et al observed that the adoption of more intensive induction protocols yielded DFS outcomes comparable to the aberrant-negative group, supporting the rationale for therapeutic intensification in aberrant-positive cases and helping in explaining the diversity of results between reports [24].

In univariate analysis for DFS , both the Pro-B subtype and aberrant lineage were linked to higher relapse risk; however, after multivariate adjustment, aberrant lineage remained the sole independent predictor of poor DFS with HR=3.37. in addition, Antigen-specific analysis revealed that while CD13 and CD33 were significant in univariate models, only CD33 remained independent, unfavorable prognostic factor for relapse with HR=2.28.

The hazard ratio of 3.37 reflects its impact and highlights the importance of routine immunophenotypic screening at diagnosis and the potential need for treatment intensification, especially for those with CD33-positive aberrant antigen expression.

5. Conclusion

Aberrant antigen expression in pediatric B-ALL remains a phenomenon of ongoing interest, with reported frequencies varying widely across studies. In our cohort, CD33 emerged as the most prevalent aberrant marker, followed by CD13. While the prognostic impact of such aberrancies has been debated, our analysis identified aberrant expression particularly CD33 positivity as an independent predictor of lower disease-free survival, with a hazard ratio of 3.37 for aberrant expression overall and 2.28 for CD33. These findings emphasize the potential need for treatment intensification in that high-risk subgroup to avoid disease recurrence and improve long-term outcome and further evaluation in larger prospective studies.

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Ethics approval: The study was reviewed and approved by Mansoura Faculty of Medicine Institutional Research Board (MD.23.07.792).

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

List of Abbreviations

ALL: acute lymphoblastic leukemia
B-ALL: B-cell acute lymphoblastic leukemia
BFM: Berlin-Frankfurt-Münster
CCG: Children's Cancer Group
CI: confidence interval
CNS: central nervous system

DFS: disease-free survival
HR: hazard ratio
IQR: interquartile range
OS: overall survival
POG: Pediatric Oncology Group
SD: standard deviation
WBC: white blood cell

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