Original Article

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Post-Induction Minimal Residual Disease Study by Flow Cytometry in Pediatric B ALL and Its Correlation with Initial Clinical and Laboratory Parameters

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is a cancer characterized by the rapid and uncontrolled growth of immature lymphoid cells. Among its subtypes, B-lineage ALL in children represents the most frequently diagnosed malignancy in the pediatric population. Despite high survival rates, relapsed or refractory cases remain challenging. Minimal Residual Disease (MRD), detected by flow cytometry, is a strong prognostic marker that guides the intensity of post-induction therapy. **Aim**: This study aims to evaluate the correlation between post-induction MRD, measured by multicolor flow cytometry, and the initial clinical and laboratory parameters in pediatric patients with B-ALL. **Methods**: A retrospective cross-sectional study was conducted at the Children's Specialty Hospital in Basrah, including 51 pediatric B-ALL patients (aged 1–14 years) treated between April 2023 and August 2024. All patients successfully completed the induction phase of chemotherapy, and their MRD status was subsequently evaluated. **Results**: MRD was negative in 60.78% and positive in 39.22% of patients. The majority of patients in both MRD groups were under 10 years old, with no significant age-related difference (P = 0.732). Patients with positive MRD had significantly higher mean white blood cell (WBC) counts (58.62 ± 86.9 vs. 11.44 ± 13.04) and blast cell percentages (87.1% ± 7.6% vs. 76.6% ± 16%) compared to those with negative MRD (P = 0.004 and 0.008, respectively). No significant associations were found between MRD and hemoglobin levels, platelet counts, or National Cancer Institute (NCI) risk classification. **Conclusions**: Flow cytometric MRD detection is a valuable prognostic tool in pediatric B-ALL. Higher WBC counts and blast percentages at presentation are associated with positive MRD, while MRD was not significantly linked to age, hemoglobin, platelet count, or risk stratification.

Keywords: B-cell acute lymphoblastic leukemia, minimal residual disease, pediatric leukemia, flow cytometry

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid precursor cells that are arrested at an early stage of differentiation. These abnormal cells can infiltrate the bone marrow, peripheral blood, and extramedullary sites. In the United States, the incidence of ALL was estimated at 1.57 per 100,000 individuals in 2014, with approximately 5,960 new cases diagnosed and 1,470 deaths reported in 2018.[1] The male-

to-female ratio is approximately 1.2:1, and the disease is more commonly observed in children.[2] Age-specific incidence is highest in children aged 1–4 years, then drops sharply through childhood (5–14 years).[2] Outcome has improved considerably over the past four decades, with an increase in the 5-year overall survival rate from 31% in 1975 to nearly 70% in 2009. However, these results conceal important disparities; although the 5-year overall survival rate reached 90% in children with acute lymphoblastic leukemia.[3] ALL is generally divided into two main subtypes based on immunophenotyping: B-cell lymphoblastic leukemia (B-ALL) and T-cell lymphoblastic leukemia (T-ALL), with B-ALL comprising approximately 85% of cases, although this percentage can differ depending on age at diagnosis, race, or ethnicity.[4] Pediatric B-lineage ALL is the most prevalent malignancy in childhood. Although well-designed clinical trials have led to excellent survival rates, patients with refractory or relapsed disease remain a therapeutic challenge.[5] In Iraq, pediatric B-lineage ALL (B-ALL) treatment protocols are primarily based on adapted UKALL (United Kingdom ALL) protocols, with some patients also treated according to modified BFM (Berlin-Frankfurt-Münster) protocols. The UKALL protocols (UKALL97/99, UKALL2003, and UKALL2011) have been used in Iraq, with the UKALL2011 protocol being implemented from 2014 onwards. A modified BFM95 high-risk protocol is used for T-ALL patients, particularly those with a mediastinal mass or positive flow cytometry.[6] Minimal Residual Disease (MRD) following therapy is one of the strongest independent prognostic markers for B-ALL.[7] MRD is evaluated at nearly all time points, including early treatment phases, during or after induction, and early consolidation. In pediatric B-ALL, MRD has become a critical tool for risk stratification, guiding the intensity of post-induction therapy.[7] MRD detection by flow cytometry relies on distinguishing leukemic cells based on their aberrant antigen expression profiles, which differ from those of normal maturing precursor cells (hematogones). Alternatively, MRD can be assessed using polymerase chain reaction (PCR), which targets and amplifies leukemia-specific DNA sequences.[8] These techniques offer high sensitivity, with flow cytometry capable of detecting one leukemic cell among 10,000 normal bone marrow mononuclear cells, and PCR able to identify one leukemic cell among 100,000 normal cells.[9] These methods are not widely utilized in low- and middle-income countries due to limited resources and expertise. Flow cytometry, however, offers advantages such as a faster turnaround time, lower cost, and reduced labor intensity. Therefore, flow cytometry-based MRD assessment holds promise for quickly identifying patients at higher risk of relapse, enabling timely adjustments in treatment, including earlier therapy intensification.[10] Although MRD has major prognostic significance, its correlation with other prognostic factors has not been thoroughly explored in pediatric ALL patients.[10] Some presenting features of ALL show an association with the rate and magnitude by which cytoreduction occurs.[11] This study aims to investigate the correlation of post-induction MRD, measured by multicolored flow cytometry, in pediatric B-ALL patients with their initial laboratory findings and clinical presentation.

MATERIALS AND METHODS

This is a retrospective, cross-sectional study carried out at the Children's Specialty Hospital in Basrah on pediatric patients (1-14 years) with B-ALL from April 2023 to August 2024. Over the defined study period, when post-induction MRD was established in the mentioned hospital, data from the available 51 patients with B-ALL who received the induction phase of chemotherapy were analyzed. The patients' data were collected from the medical records of each patient diagnosed with B-ALL at the hospital and a private laboratory database. Data extraction was performed using a standardized form, and two independent reviewers cross-verified the information to enhance accuracy and reduce the potential for misclassification bias. Demographic data regarding age and sex, as well as clinical data such as fever, lymphadenopathy, and hepatosplenomegaly by imaging, were recorded for each patient. Hematological data taken at the presentation of each patient included total leukocyte count (TLC), hemoglobin (Hb), and platelet count using the hematological analyzer SYSMEX XN-350 (Kobe, Japan). All B-ALL patients with post-induction MRD were included in the study. Day 35 was chosen as the time point for MRD assessment in line with widely accepted international treatment protocols for pediatric ALL, where this timing has been shown to correlate with early treatment response and long-term prognosis. Bone marrow aspirate samples were analyzed using multicolor flow cytometry (BD FACSLyric, 3-laser, 8-color system). The panel of monoclonal antibodies used for MRD detection included CD10, CD19, CD20, CD34, CD38, CD45, CD58, and CD66c, selected based on the initial immunophenotype of leukemic blasts. B-ALL MRD

can be roughly categorized into negative (MRD < 0.01%) and a minimum of 0.01% of blast cells classifying them as MRD positive. [12] The National Cancer Institute (NCI) used patients' age and TLC to determine risk stratification. High Risk (HR) constitutes patients aged 10 years or older and/or a TLC equal to or greater than 50,000/µL. Standard risk (SR) includes patients younger than 10 years and with a TLC count less than 50,000/µL.[13] Although socioeconomic status, treatment protocol variation, and genetic subtypes (such as cytogenetic or molecular abnormalities) may influence MRD and outcomes, these potential confounding factors were not assessed in this study due to limitations in data availability. Agreements from the Ethical Committee of Basrah Health Directorate and the Scientific Ethical Committee at the Scientific Council of Pathology of the Iraqi Board for Medical Specializations on carrying out this study were acquired before data collection (Issue No. path68, date: 5/6/2024). Written informed consent was obtained from parents, with assent from children when appropriate. The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 21. Descriptive statistics (frequency and percentage) were used to describe the studied groups, while for the quantitative data, means, standard deviations, and range were reported. Chi-square statistics were used to describe the association for categorical data, and Fisher's exact test was used when one or more cells had an expected frequency of less than five. A p-value of <0.05 was considered statistically significant.

RESULTS

The age of the studied patients ranged from 1 to 14 years, with a mean of 6.5 years ± 3.5 SD. Most of the studied patients were younger than 10 years (78.4%) (Table 1). More than half of the patients were female (approximately 28, or 54.9%), while the remaining 23 (45.1%), were male.

The mean level of hemoglobin among the studied population was 8.12 ± 2.57 g/dL, while the mean total WBC Count was 29.57 ± 59.27, and the mean platelet count was 75.05 ± 87.108. The blast Cell count was 80.73% ± 14.1%. (Table 2)

Most of the patients had the common ALL subtype (90.2%). More than half of them had negative MRD at the end of induction (60.8%). There was no significant relationship between the MRD result and ALL subtypes (P = 0.621). (Table 3)

The mean age for positive MRD was 3.5 ± 6.13.5 ± 6.1 years, compared to the mean age for negative MRD, which was 7 ± 3.6;7 ± 3.6 however, there was no statistically significant relationship (P = 0.366). In terms of sex distribution, most of the males had positive MRD (65.2%), while more than half of the females had positive MRD (57.1%) (Table 4). In this study, 82.4% of the patients were suffering from fever, while 47.1% had lymphadenopathy, and 39.2% had hepatomegaly (Table 5). This study showed that the median count/IQR of WBC and mean blast cell percentage was significantly higher (Table 6). In this study, patients younger than 10 years constituted the majority in both negative and positive MRD. MRD results; however, there was no significant statistical difference between the two age groups, P value = 0.732). In terms of WBC count, about 40% of positive MRD patients had a total WBC count of 50,000 or more. This was statistically significant (Table 7). In our study, the majority of patients were classified as standard risk according to NCI in both negative (96.8%) and positive (Table 8).

Table 1: Age and sex distribution of the studied patients

Characteristics		Frequency	Percentage
Age (years)	Less than 10	40	78.4%
	10 and older	11	21.6%
	Mean ± SD	6.5 ± 3.5	
	Minimal-Maximal	1-14	
Sex	Female	28	54.9%
	Male	23	45.1%

Table 2: Hematological parameters of the studied patients		
Hematological Parameter	Mean ± SD	Minimal-maximal
Hb (g/dL)	8.12 + 2.57	2.3-15.4
Platelet (*10^3 /UL)	75.05+ 87.108	2-497
WBC (*10^3 UL)	29.57+ 59.27	1.35-338.3
Blast (%)	80.73% ± 14.1%	37-98

Table 3: Association between MRD and ALL subtypes					
		MRD		Total	P-value
		Negative	Positive		
Subtype	Common	29 (63.0%)	17 (37.0%)	46 (100%)	0.621
	Pro-B	1 (33.3%)	2 (66.7%)	3 (100%)	
	Pre-B	1 (50.0%)	1(50.0%)	2 (100%)	
Total		31 (60.8%)	20 (39.2%)	51 (100%)	

Table 4: Association between MRD and demographic variables				
Characteristics		MRD		P-value
		Positive	Negative	
Age (years)	Mean ± SD	3.5+6.1	7+3.6	0.366
	Minimum-Maximum	1-13.2	1.3-15	
Sex	Male (%)	15 (65.2%)	8 (34.8%)	0.580
	Female (%)	16 (57.1%)	12 (42.9%)	

Table 5: Association between MRD and clinical signs					
Clinical Signs		MRD		Total	p- value
		Negative	Positive		
Fever	No	5	4	9 (17.6%)	0.724
	Yes	26	16	42 (82.4%)	
Lymphadenopathy	No	18	9	27 (52.9%)	0.402
	Yes	13	11	24 (47.1%)	
Hepatomegaly	No	19	12	31 (60.8%)	0.927
	Yes	12	8	20(39.2%)	
Splenomegaly	No	17	10	27 (52.9%)	0.780
	Yes	14	10	24(47.1%)	

Table 6: Association between MRD and hematological parameters at presentation

Blood Picture Findings		MRD		p- value
		Negative	Positive	
Hemoglobin (g/dL)	Mean ± SD	± 8.3 2.9	7.8 ± 2.1	0.483
	Minimum-maximum	2.3-15.4	3.9-11.2	
Platelet Count (*10^3 UL)	Median/IQRMedian/IQR	64.00/15	38.00/6	0.182
	Minimum-maximum	2-497	10-200	
WBC count (*10^3 UL)	Median /IQR	7.00/4.08	16.72/54.83	0.004
	Minimum-maximum	1.8-66.9	1.35-338.3	
Blast cell	Mean ± SD	76.6% ± 16%	87.1% ± 7.6%	0.008
	Minimum-maximum	37%-98%	65%-96%	

Table 7: Association between MRD and risk factors

Variable			MRD		p- value
			Negative	Positive	
AGE	Less than 10 years	Count	25	15	0.732
		%	80.6	75	
	10 years and older	Count	6	5	
		%	19.4	25	
WBC	Less than 50,000/µL	Count	30	12	0.001
		%	96.8	60	
	50,000/µL and more	Count	1	8	
		%	3.2	40	
Total		Count	31	20	
		%	100%	100%	

Table 8: Relationship between risk group and MRD result

		MRD			p- value
		Negative	Positive	Total	
Risk Group	High risk	1(33.3%)	2 (66.7%)	3(100%)	0.621
	Standard Risk	30(62.5%)	18(37.5%)	48(100%)	
Total		31(60.8%)	20(39.2%)	51(100%)	

DISCUSSION

MRD is a strong predictor of relapse in children with ALL. Its assessment correlates closely with treatment response and outcomes, serving as a quantitative supplement to conventional remission and relapse indicators rather than a replacement for initial risk factors. In this study, most patients were under 10 years old (78.4%), and slightly more were female (54.9%). Compared to Meraj et al.[12], where 71% were under 10 years and 60% were male, our age distribution is similar, but our sex distribution differs. We found that 39.2% of patients had positive MRD at the end of induction. This is higher than the 12% reported by Katsibardi et al.[13] 28.6% by Borowitz et al.[14], and similar to the 37% reported by Meraj et al.[12] The higher MRD rate in our cohort may reflect socioeconomic challenges such as poor nutrition, delayed diagnosis, and limited access to supportive care.[12] Data from Tembhare et al. demonstrated that patients with post-induction MRD levels ≥0.01% had

poor clinical outcomes. Interestingly, even a small subset of patients (n = 17) with MRD levels <0.01% showed unfavorable outcomes. This unexpected finding may be attributed to the underestimation of MRD levels, potentially caused by common practical challenges such (90%) MRD results showed no significant association between MRD results and risk stratification among the studied patients (P = 0.621), hemodilution, limited sample cellularity, or inadequate specimen quality may have contributed to this outcome.[15] Although MRD positivity was more frequent among males (65.2%) than females (57.1%), the association was not statistically significant (P = 0.580), consistent with Meraj et al.[12] who also found no significant sex-based difference (P = 0.217). Similarly, MRD positivity was more common in patients under 10, again with no significant association—matching findings by Meraj et al.[12] and Yamaji et al.[16] A significant relationship was found between MRD positivity and high initial WBC count (≥50,000/µL). Zhou et al.[17] reported similar results (28.9% with high WBC), while Meraj et al.[12] found no such association. Farweez et al.[18] also noted higher TLC and blast counts in MRD-positive cases, although hemoglobin differences were not significant. Elevated TLC at diagnosis is a known risk factor for poor outcomes.[19] The NOPHO group identified hyperleukocytosis as a high-risk factor negatively affecting 5-year event-free survival in patients with B-ALL.[20] Similarly, a study by Petit et al. demonstrated that elevated WBC counts (≥200 × 10^9/L) were associated with a poor prognosis in T-cell acute lymphoblastic leukemia.[21] Most of our patients (94.1%) were classified as standard risk based on NCI criteria. There was no significant relationship between MRD status and risk category (P = 0.621). In contrast, Meraj et al.[12] reported a higher proportion of MRD-positive cases among high-risk patients (59%), but with no statistical significance (P = 0.077). However, Farweez et al.[18] and Zhou et al.[17] found strong associations between MRD positivity and high-risk classification (P < 0.001), highlighting the prognostic relevance of NCI risk stratification in other settings.

CONCLUSIONS

Flow cytometry-based MRD detection is a valuable prognostic tool in pediatric B-ALL. In this study, 60.78% of patients were MRD-negative and 39.22% were MRD-positive. MRD status showed no significant association with age, sex, NCI risk, or clinical signs. However, WBC count and blast percentage were significantly higher in MRD-positive patients, while hemoglobin and platelet levels showed no correlation.

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