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Post-induction measurable residual disease evaluation in pediatric B-acute lymphoblastic leukemia and its related factors; a prospective observational study based on flow cytometry

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Abstract

Introduction: B-acute lymphoblastic leukemia (B-ALL) is the most common pediatric malignancy. Despite significant advancements in treatment protocols, relapse remains a major challenge. Measurable residual disease (MRD), defined as the presence of leukemic cells below the detection threshold of conventional microscopy, has emerged as a robust prognostic marker.

Objectives: This study investigates post-induction MRD levels in pediatric patients with B-ALL and examines factors influencing MRD positivity.

Patients and Methods: This prospective observational study employed multi-parametric flow cytometry (MFC) to evaluate MRD levels in 38 pediatric B-ALL patients following induction therapy referred to Nanakali hemato-oncology center in Erbil, Iraq, between June 2023 and June 2024. The cohort included children aged 1–15 years diagnosed with B-ALL. Bone marrow (BM) samples were collected at the end of induction therapy, since MRD was quantified using a flow cytometry protocol. Clinicopathological data were analyzed to identify factors associated with MRD status.

Results: Out of 38 B-ALL patients, 11 (28.9%) were MRD-positive and 27 (71.1%) were MRD-negative. In the unadjusted analysis, a white blood cell (WBC) count exceeding 50×10^9/L was strongly associated with MRD positivity, with an odds ratio (OR) of 6.90, indicating a significantly higher likelihood of MRD detection. In contrast, hemoglobin (Hb) levels demonstrated a protective effect; for every 1 g/dL increase in Hb, the odds of MRD positivity decreased by 43%, corresponding to an OR of 0.57. Additionally, BM blast percentages on day 8 and post-induction (day 35) were significantly correlated with MRD positivity, with ORs of 12.57 and 5.12, respectively. However, age, gender, baseline symptoms, lymphadenopathy, organomegaly, symptom duration, and cytogenetic abnormalities, platelet count, peripheral blood blasts, treatment type, and lactate dehydrogenase (LDH) levels showed no statistically significant correlation with MRD status.

Conclusion: Our study findings highlight the importance of WBC count, BM blast percentages, and Hb levels in risk stratification of MRD positivity, suggesting targeted interventions for high-risk patients could improve outcomes.

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Introduction

Acute lymphoblastic leukemia (ALL) is a highly aggressive hematological malignancy characterized by the uncontrolled proliferation and accumulation of immature lymphoid precursor cells within the bone marrow (BM) and peripheral blood. This abnormal buildup disrupts normal hematopoiesis, leading to a deficiency in healthy blood cells and resulting in clinical manifestations such as anemia, thrombocytopenia, and increased susceptibility to infections (1).

This malignancy primarily affects children but can also occur in adults (2). In the United States, the incidence of ALL has risen by 2.2% over the past decade (3,4). Similarly, in Iraq, leukemia was the most common childhood cancer between 2000 and 2019, accounting for 32.96% of cases, with an annual increase of 1.23% (5).

The therapy of ALL has evolved dramatically throughout the years, with an emphasis on improving outcomes and reducing relapse rates. However, relapse can still occur even after utilizing a risk-adapted therapy and attaining a maximum response rate (6). These relapses occur because small numbers of leukemic blasts persist in the body at a too low levels to be detected by standard diagnostic techniques. However, highly

Key point

In a prospective observational study, we identified significant correlations between measurable residual disease (MRD) positivity and three key factors such as white blood cell (WBC) count, hemoglobin (Hb) levels, and bone marrow (BM) blast percentage. Conversely, other clinicopathological parameters, including age, gender, baseline symptoms, lymphadenopathy, organomegaly, symptom duration, cytogenetic abnormalities, platelet count, peripheral blood blasts, treatment type, and lactate dehydrogenase (LDH) levels, did not show a statistically significant association with MRD status.

sensitive techniques like multiparameter flow cytometry (MFC), polymerase chain reaction (PCR), and high-throughput next generation sequencing can detect these residual leukemic cells in about 30-50% of patients who achieved complete remission. These persistent leukemic cells in the context of complete remission are referred to as the measurable residual disease (MRD). Detecting and monitoring MRD levels is critical for assessing the effectiveness of treatment, predicting the risk of relapse, and guiding subsequent treatment strategies (7,8).

The new flow cytometry that uses ≥8-color has increased the sensitivity to detect residual leukemia cells in 1 out of 100 000 cells when acquiring sufficient cells, making it equivalent to the PCR approach, since it is now widely utilized for ALL cases due to its more applicability, time-bound and less cost (9). MFC is conducted to detect MRD by using an array of antibodies to assess the aberrant expression of leukemia-associated immunophenotype which includes either reduced expression of the markers or increased expression of the markers normally expressed by benign B-cells or aberrant expression of myeloid markers. Another approach used for MRD assessment by flow cytometry is the 'different-from-normal' method that does not require the initial diagnostic sample as it depends on the difference of immunophenotypes from the standard normal distribution; however, a combined leukemia-associated immunophenotype based 'different-from-normal' approach is now recommended to assess MRD in acute leukemia (10,11). MRD can be used to evaluate residual leukemia at any time point; however, it is most typically performed after the end of induction chemotherapy and is regarded as a powerful predictor of outcome (12,13). Due to the limited evidence on the use of MRD evaluation of B-ALL patients in Iraq, this study aimed to assess post-induction MRD in pediatric patients with B-ALL to explore its relationship with clinicopathological factors, treatment intensification, and patient outcomes.

Objectives

This study aims to evaluate post-induction MRD levels in pediatric patients with B-ALL using multiparametric flow cytometry and to identify clinicopathological factors associated with MRD positivity, thereby providing insights into prognostic implications and guiding risk-adapted therapeutic strategies.

Materials and Methods

Study design and participants

This prospective observational study was conducted at Nanakali hemato-oncology center, a specialized facility for blood diseases and cancer in Erbil, Iraq, between June 2023 and June 2024. The study enrolled 38 pediatric patients with B-ALL who had undergone induction chemotherapy. Ethical approval for the study was obtained from the ethics committee of Hawler medical university, ensuring compliance with relevant ethical standards.

Inclusion and exclusion criteria

The inclusion criteria for this study consisted of pediatric patients with B-ALL aged 15 years or younger, as per the admission guidelines of our tertiary center, who had undergone induction therapy and were referred for MRD evaluation. Conversely, patients older than 15 years and those who were lost to follow-up were excluded from the study, resulting in the exclusion of nine patients.

Data collection

Following written informed consent from the patients' guardians, comprehensive demographic data, including age and gender, along with detailed medical histories and reported symptoms, were collected from all participants. Baseline laboratory results were retrieved from patient records, encompassing hemoglobin (Hb) levels, white blood cell (WBC) counts, platelet counts, serum lactate dehydrogenase (LDH) levels, blast percentages in both peripheral blood and BM, immunophenotypic markers, cytogenetic and genetic analyses when available, and the specific treatment protocols administered. Event-free survival (EFS) was measured from the start of therapy to the occurrence of an event, while overall survival (OS) was calculated from the initiation of therapy to the date of death.

Procedure

The diagnosis of B-ALL in this study relied on both morphological and immunophenotypic characteristics. Treatment was guided by the United Kingdom ALL 2011 (UK-ALL11) protocol, with intensification as necessary (14). For each patient, a 3 ml BM aspirate sample was collected into an EDTA tube on day 35 post-induction. This sample was conducted for morphological assessment and flow cytometry-based evaluation of MRD status. The MRD assessment was performed using a multicolor flow cytometer (BD FACSCanto II), which features three lasers and an eight-color platform, allowing for precise detection and characterization of residual leukemic cells. All samples were processed within two hours of collection to ensure optimal preservation of cellular integrity (15). For each sample, two tubes were prepared according to the EuroFlow panel (16). Samples were acquired using BD FACSDiva software, ensuring a minimum of two million events per tube to ensure robust data quality. Daily

quality control and compensation were performed using BD FACS™ calibration beads to maintain instrument calibration. Cell populations were identified by leveraging the forward scatter and side scatter properties, combined with fluorescence intensity analysis using specific antibody combinations.

Treatment for the patients in this study was administered according to the UK-ALL11 protocol, with treatment intensity tailored based on risk stratification and MRD status (14). MRD positivity was defined as a level of ≥0.01%, while levels below 0.01% were considered negative (17). Morphologic complete remission was achieved when BM blasts were less than 5%, with no evidence of extra-medullary leukemia, and when the absolute neutrophil count exceeded 1.5 × 10^9/L and the platelet count exceeded 100 × 10^9/L (18).

Outcomes

The primary outcome is to determine the MRD status post-induction therapy. Secondary outcomes include assessing the correlation between MRD status and clinicopathological features such as age, gender, symptoms, laboratory findings, and cytogenetic abnormalities. Additionally, the study evaluates how MRD status relates to treatment response and survival outcomes. Ultimately, the study seeks to enhance understanding of MRD in pediatric B-ALL, informing personalized treatment approaches to improve patient outcomes.

Statistical analysis

Statistical analysis was conducted using SPSS version 27. The normality of quantitative data was assessed via the Shapiro-Wilk test and visual inspection using Q-Q plots. Non-normally distributed data were summarized as median and interquartile range (IQR). The Mann-Whitney U test was employed to compare numerical data between groups. Categorical variables were expressed as frequencies (percentages) and analyzed using the chi-square test and Fisher's exact test to evaluate associations. Logistic regression was used to explore the correlation between MRD status and clinicopathological factors. The ideal cut-off thresholds for positive MRD were established through the analysis of receiver operating characteristic (ROC) curves, utilizing the area under the curve (AUC) to evaluate its predictive efficacy concerning MRD status. Furthermore, essential diagnostic parameters, encompassing sensitivity and specificity, were calculated for the optimal cut-off thresholds. Survival outcomes were evaluated using the Kaplan-Meier method, with events defined as relapse or death, and the log-rank test was applied to compare differences between MRD-positive and MRD-negative groups. The EFS was measured from the start of therapy to the occurrence of an event, while OS was calculated from the initiation of therapy to the date of death. Statistical significance was defined as a *P* value of ≤0.05.

Results

This study included 38 pediatric patients diagnosed with B-ALL. The median age of the participants was 5.5 years, ranging from 1 to 15 years. The study population consisted of a slightly higher proportion of males, accounting for 57.9% of the patients, while females comprised 42.1%. Regarding MRD status, 28.9% of the patients were MRD-positive, whereas the majority, 71.1%, were MRD-negative. The frequency distributing evaluation of demographic factors and clinical data between groups with positive and negative MRD status revealed no statistically significant differences in most parameters, including age, gender, baseline symptoms (such as fever, pallor, easy fatigability, pain, bleeding, weight loss), lymphadenopathy, organomegaly, symptom duration, and cytogenetic abnormalities. However, the WBC distribution was significant, and also the type of treatment administered showed a highly significant difference between the two groups, with a total use of the intensified method in the positive MRD group (Table 1).

The analysis of laboratory and BM data between groups with positive and negative MRD status revealed significant differences in specific parameters. Hemoglobin levels, BM blasts on day 8, and BM blasts post-induction (day 35) were significantly associated with MRD status. Conversely, other parameters, including platelets count, peripheral blood blasts, BM blasts at baseline, and LDH levels, did not show statistically significant differences between the two groups (Table 2).

Table 3 explored the association between positive MRD status and several clinical variables using logistic regression. In the unadjusted analysis, a WBC count exceeding 50 × 10^9/L was significantly linked to positive MRD, with an odds ratio (OR) of 6.90, demonstrating a substantially elevated likelihood of MRD positivity. Conversely, Hb levels exhibited a protective effect; for each g/dL increase in Hb, the odds of positive MRD decreased by 43% (OR = 0.57). BM blast percentages on day 8 and post-induction (day 35) also showed significant associations with positive MRD, with ORs of 12.57 and 5.12, respectively. This suggests that for each percentage point increase in BM blasts at day 8 and post-induction, the risk of positive MRD increased by 12.57 and 5.12 times, respectively. However, after adjusting for potential confounders, none of these associations remained statistically significant, and all were found to be non-independent predictors (Table 3).

Table 4 assessed the diagnostic value of several parameters in predicting positive MRD. Hb levels demonstrated limited diagnostic utility, whereas BM blast percentages on day 8 and post-induction (day 35) exhibited better performance, with AUC values indicating moderate accuracy. Specifically, using a cutoff of 2.5%, BM blasts on day 8 showed a high sensitivity of 88% and moderate specificity of 62% for predicting positive MRD. Similarly, with the same cutoff, BM blasts post-induction displayed a slightly lower sensitivity of 82% but improved

Table 1. Frequency distribution of the basic and clinical characteristics of B-ALL patients based on MRD status

Parameter		MRD			*P* value
		Positive (n = 11)	Negative (n = 27)	Total (N = 38)	
		No. (%)	No. (%)	No. (%)	
Age (y)	<10	7 (63.6)	21 (77.8)	28 (73.7)	0.432*
	≥10	4 (36.4)	6 (22.2)	10 (26.3)	
Gender	Male	8 (72.7)	14 (51.9)	22 (57.9)	0.296*
	Female	3 (27.3)	13 (48.1)	16 (42.1)	
Symptoms at baseline	Fever	9 (81.8)	14 (51.9)	23 (60.5)	0.145*
	Pallor	9 (81.8)	16 (59.3)	25 (65.8)	0.268*
	Easy fatigability	6 (54.5)	11 (40.7)	17 (44.7)	0.491*
	Pain	5 (45.5)	9 (33.3)	14 (36.8)	0.712*
	Bleeding	3 (27.3)	4 (14.8)	7 (18.4)	0.390*
	Weight loss	5 (45.5)	5 (18.5)	10 (26.3)	0.116*
Lymphadenopathy		6 (54.5)	13 (48.1)	19 (50.0)	0.721*
Organomegaly		8 (72.7)	17 (63.0)	25 (65.8)	0.714*
Symptoms Duration (wk)	<2	0 (0.0)	4 (14.8)	4 (10.5)	0.459**
	2-4	11 (100.0)	21 (77.8)	32 (84.2)	
	>4	0 (0.0)	2 (7.4)	2 (5.3)	
Cytogenetic	Normal karyotype	4 (44.4)	10 (50.0)	14 (48.3)	0.280**
	Hyperdiploidy	3 (33.3)	5 (25.0)	8 (27.6)	
	t(12,21)	0 (0.0)	3 (15.0)	3 (10.3)	
	MLL rearrangement	0 (0.0)	1 (5.0)	1 (3.4)	
	Abnormal p 17	2 (22.2)	0 (0.0)	2 (6.9)	
	Complex karyotype	0 (0.0)	1 (5.0)	1 (3.4)	
WBC	<50 (×10⁹/L)	23 (85.2)	5 (45.5)	28 (73.7)	0.012*
	>50 (×10⁹/L)	4 (14.8)	6 (54.5)	10 (26.3)	
Treatment	Maintained	0 (0.0)	20 (74.1)	20 (52.6)	<0.001*
	Intensified	11 (100.0)	7 (25.9)	18 (47.4)	

B-ALL, B-acute lymphoblastic leukemia; MRD, Measurable residual disease; WBC, white blood cells. *Chi-square, **Fishers' exact test.

Table 2. Frequency distribution of the laboratory data and bone marrow analysis of B-ALL patients according to MRD status

Parameter	MRD			*P* value*
	Positive Median (IQR)	Negative Median (IQR)	Total Median (IQR)	
Hb (g/dL)	7.6 (5.6-10.0)	8.9 (5.8-11.0)	8.6 (5.6-11.0)	0.030
Platelets (×10⁹/L)	45.0 (7.0-96.0)	52.0 (6.0-142.0)	50.0 (6.0-142.0)	0.350
PB blasts (%)	57.0 (4.0-84.0)	50.0 (0.0-85.0)	54.4 (0.0-85.0)	0.821
BM blasts (%)	80.0 (54.0-90.0)	75.0 (30.0-90.0)	80.0 (30.0-90.0)	0.443
BM blasts on day 8 (%)	3.0 (2.0-3.0)	2.0 (2.0-3.0)	3.0 (2.0-3.0)	0.016
BM blasts post induction (day 35) (%)	3.0 (2.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.007
LDH (IU/L)	665.0 (412.0-1371.0)	625.0 (312.0-2616.0)	649.5 (312.0-2616.0)	0.840

B-ALL, B-acute lymphoblastic leukemia; Hb, hemoglobin; MRD, Measurable residual disease; IQR, Interquartile range; PB, Peripheral blood; BM, Bone marrow; LDH, Lactate dehydrogenase. *Mann-Whitney U test.

specificity of 71% in predicting positive MRD. These findings suggest that BM blast percentages, particularly at day 8 and post-induction, may serve as useful indicators for predicting positive MRD, given their ability to identify a substantial proportion of positive cases, although with a moderate rate of false positives (Table 4 and Figure 1).

The median follow-up duration for the patients spanned from 1 to 12 months, with a median of 6 months. During this period, eight patients experienced relapses, with four of these cases having positive MRD. Furthermore, five patients succumbed to their illness, and three of these had positive MRD. The OS and EFS rates at one year for the entire cohort were 84.45% and 40.6%, respectively. Notably, the one-year OS for patients with positive MRD

Table 3. The correlation between MRD and clinicopathological parameters using binary logistic regression

Variable		Positive MRD							
		Unadjusted				Adjusted			
		OR	*P* value	95% CI		OR	*P* value	95% CI	
				Lower	Upper			Lower	Upper
WBC (×10⁹/L)	<50	Reference							
	>50	6.90	0.017	1.40	33.91	2.61	0.472	0.19	35.78
Treatment	Maintained	Reference							
	Intensified	-	0.998	-	-				
HB (g/dl)		0.57	0.043	0.33	0.98	0.52	0.159	0.21	1.28
BM blasts on day 8 (%)		12.57	0.030	1.28	123.48	2.18	0.659	0.06	70.37
BM blasts post induction (day 35) (%)		5.12	0.022	1.26	20.75	5.41	0.328	0.18	159.41

WBC, White blood cell; Hb, hemoglobin; MRD, Measurable residual disease; BM, Bone marrow; OR, Odds ratio; CI, Confidence interval.

Table 4. Diagnostic value of Hb, BM blasts on day 8, and BM blasts post induction (day 35) in predicting positive MRD

Parameter	Positive MRD diagnostic value						
	AUC (0-1)	*P* value	95% CI		Cut of	Sensitivity (%)	Specificity (%)
			Lower	Upper			
Hb (g/dL)	0.273	0.030	0.104	0.442	7.5	55	23
BM blasts on day 8 (%)	0.750	0.037	0.559	0.941	2.5	88	62
BM blasts post induction (day 35) (%)	0.756	0.014	0.594	0.918	2.5	82	71

AUC, Area under curve; Hb, hemoglobin; MRD, Measurable residual disease; BM, Bone marrow; CI, Confidence interval.

was significantly lower compared to those with negative MRD, at 68.2% versus 75.0%, with a statistically significant difference as indicated by a log-rank test p-value of 0.022. The one-year EFS rates differed significantly between the MRD-positive and MRD-negative groups. Specifically, patients with positive MRD had a notably lower one-year EFS rate compared to those with negative MRD. This disparity was statistically significant, as confirmed by a log-rank test with a *P* value of 0.016, highlighting the adverse impact of positive MRD on survival outcomes (Figures 2 and 3).

Discussion

In our study, 28.9% of a population of 38 B-ALL patients were found to be MRD-positive. A finding closely aligned with ours was reported by Borowitz et al, who observed a 27.7% incidence of post-induction MRD positivity (19). However, the majority of studies have documented higher rates of post-induction MRD positivity; a study conducted by Panda et al found that 40.2% of ALL patients under the age of 25 in India had MRD positivity on the 35th day following induction therapy (7). Balasubramanian et al reported that 21.7% of pediatric patients showed MRD positive (20). Tembhare et al reported an incidence of 43.2 MRD positive among B-ALL pediatric patients (21). In a study, the MRD positivity rates in post-induction phases have been reported from 30% to 50% in adult B-ALL cohorts (22). A pediatric study reported MRD positivity rates of 37% post-induction and 44% post-consolidation (23). These variations may reflect differences in patient

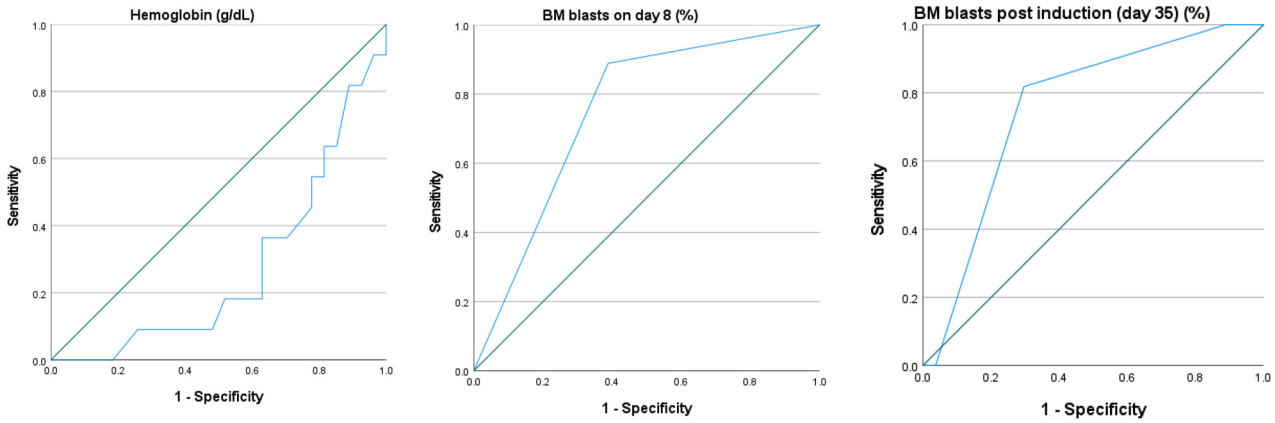

Figure 1. Diagnostic value of hemoglobin, BM blasts on day 8, and BM blasts post induction (day 35) in predicting positive MRD using ROC curve analysis.

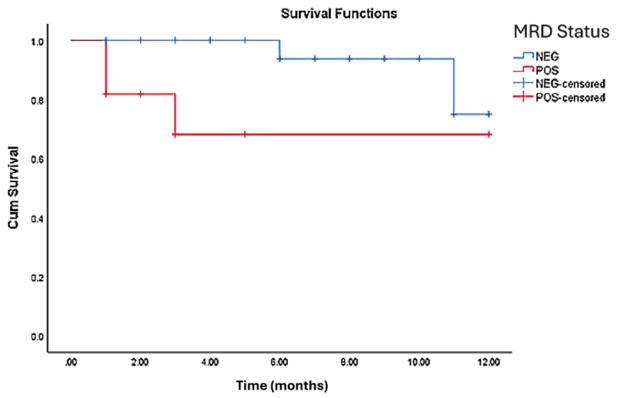

Figure 2. Kaplan–Meier survival analysis of overall survival in MRD-negative (blue line) and MRD-positive (red line) B-ALL patients.

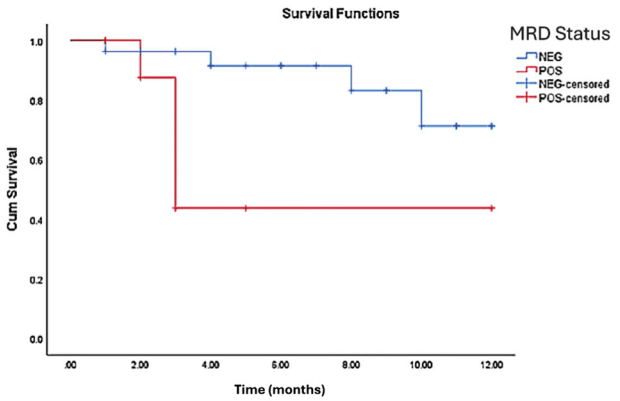

Figure 3. Kaplan–Meier survival analysis of event free survival in MRD-negative (blue line) and MRD-positive (red line) B-ALL patients

populations, timing of MRD assessment, or sensitivity of detection techniques. Therefore, our relatively lower incidence could be explained by a combination of factors, such as the small number of patients included in this study, the possibility of diluted BM aspirate samples used for MRD detection despite the bulk lysis method used, or it may be due to the lower acquired events in comparison to other studies.

The study also found that among the factors analyzed, WBC count, Hb levels, and BM blast percentage emerged as the most commonly associated variables with MRD positivity; and MRD-positive patients had significantly poorer OS and EFS than the negative group. Panda et al conducted a study that revealed no statistically significant differences in outcomes between MRD-positive and MRD-negative patients, despite observing poorer OS and EFS trends in the MRD-positive group. Specifically, the 5-year EFS rates were 69% for MRD-negative patients compared to 61.1% for MRD-positive patients. Similarly, the 5-year OS rates were 72.5% for MRD-negative patients versus 61.1% for MRD-positive patients (7). In line with our study, the study conducted by Hay et al demonstrated a significant correlation between MRD status and clinical outcomes in patients. Specifically, those who achieved MRD-negative status exhibited substantially improved EFS and OS compared to their MRD-positive counterparts (24). This finding underscores the prognostic value of MRD negativity, suggesting that it is a critical indicator of long-term clinical success. By achieving MRD negativity, patients can experience enhanced outcomes, highlighting the importance of monitoring and managing residual disease in treatment protocols.

Previous studies also demonstrated that MRD positivity in BM or peripheral blood is linked to worse survival outcomes across various leukemias, including acute myeloid leukemia (AML) and ALL. Specifically, BM MRD positivity has been shown to predict inferior OS and EFS in pediatric AML patients, even when peripheral blood MRD is negative at early time points (25,26). Similarly, achieving MRD negativity has been associated with significantly improved OS and progression-free survival in both younger and older AML cohorts, as well as in other hematologic cancers (27,28). Overall, these findings underscore the critical role of MRD status as a biomarker for risk stratification and treatment optimization. The current study further reinforces the need for integrating MRD monitoring into clinical protocols to refine prognostic assessments and guide therapeutic decisions.

Conclusion

The findings indicate that nearly 29% of patients were MRD-positive, underscoring the need for precise monitoring to guide treatment strategies. Notably, elevated WBC counts and higher BM blast percentages were strongly associated with MRD positivity, suggesting these factors as significant predictors of residual disease. Conversely, higher Hb levels appeared to have a protective effect against MRD positivity. However, other clinical and laboratory parameters such as age, gender, baseline symptoms, and cytogenetic abnormalities did not show a significant correlation with MRD status. These results emphasize the role of Hb, WBC count, and BM blast percentages in risk stratification and suggest that targeted interventions may be beneficial for patients with these high-risk features.

Limitations of the study

It is an observational study, which cannot establish causality and is susceptible to confounding biases. The sample size of 38 patients is relatively small, potentially limiting the generalizability of the findings. The study was conducted at a single center in Erbil, Iraq, which may not be representative of diverse populations or healthcare settings globally. Additionally, the study lacks long-term follow-up data, limiting the assessment of how post-induction MRD status influences long-term outcomes such as relapse rates and survival. Finally, there is a risk of selection bias if the study population does not accurately reflect the broader population of pediatric B-ALL patients, affecting the validity and generalizability of the results.

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Authors' contribution

Conceptualization: Sarah Layth Alnuaimy and Hiwa Hassan Hamza.
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Formal analysis: Nawsherwan Sadiq Mohammad.
Investigation: Mohammed Jarjees Hashm and Nawsherwan Sadiq Mohammad.
Methodology: Mohammed Jarjees Hashm and Nawsherwan Sadiq Mohammad.
Project management: Sarah Layth Alnuaimy.
Resources: All authors.
Supervision: Hiwa Hassan Hamza.
Validation: Hiwa Hassan Hamza.
Writing–original draft: All authors.
Writing–reviewing and editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

The research was conducted in accordance with the principles outlined in the Declaration of Helsinki. Written informed consent was taken from all participants. This study resulted from a research project approved by the ethics committee of Hawler Medical University, College of Medicine, Erbil, Iraq (Ethical code #21). Besides, the authors have ultimately addressed ethical standards including issues related to plagiarism, data fabrication, and duplicate publication.

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