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Original Research Article

Assessment of measurable residual disease after induction chemotherapy in acute lymphoblastic leukemia patients in a tertiary care hospital

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ABSTRACT

Background: Measurable residual disease (MRD) is a crucial prognostic factor in acute lymphoblastic leukemia (ALL), influencing treatment outcomes and long-term survival. This study investigates the prevalence of MRD post-induction chemotherapy and its association with clinical, demographic, and biochemical markers in ALL patients at a tertiary care hospital.

Methods: This observational cross-sectional study was conducted at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, from August 2021 to July 2022. A total of 22 newly diagnosed ALL patients underwent induction chemotherapy and MRD evaluation using flow cytometry. Clinical signs, demographic data, and laboratory findings, including serum creatinine, uric acid, and BCR-ABL1 status, were recorded. Statistical analyses assessed the relationships between MRD status and various predictors.

Results: Of the 22 patients, 7 (31.82%) remained MRD positive after induction chemotherapy. Anaemia was prevalent, affecting 20 participants (90.91%). MRD positive patients had higher serum creatinine (mean 1.23±0.31 mg/dl) and uric acid levels (mean 7.09±1.62 mg/dl) compared to MRD negative patients. None of the MRD positive patients tested positive for BCR-ABL1. Six of the 7 MRD positive patients were male (85.71%). Bone marrow analysis showed 5 MRD positive patients (71.43%) achieved complete remission. Risk stratification did not significantly correlate with MRD status.

Conclusions: MRD is a critical prognostic tool in ALL management, correlating with higher biochemical markers of tumor burden and a distinct clinical profile. These findings support integrating MRD assessment with detailed clinical and laboratory evaluations to refine treatment strategies and improve patient outcomes in ALL.

Keywords: Acute lymphoblastic leukemia, Measurable residual disease, Induction chemotherapy, Clinical outcomes, Biochemical markers

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is characterized by the malignant transformation and rapid proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites. This cancer predominantly affects children, who account for 80% of ALL cases, but it also presents a significant health challenge in adults, where the prognosis is generally poorer.[1,2] Despite advancements in treatment protocols that have pushed survival rates to 90% in high-income countries, survival in regions like India remains lower at around 60%, with relapse being a major cause of mortality.[3-5] Measurable residual disease (MRD), previously known as minimal residual disease, is a potent prognostic indicator in ALL management. MRD refers to the presence of leukemia cells post-treatment that cannot be detected by conventional microscopy.[6] Studies indicate that MRD positivity correlates strongly with an increased risk of relapse; patients with MRD levels below 0.01% post-therapy have significantly better outcomes than those with higher levels.[7] MRD's pivotal role extends across all ALL subtypes, regardless of the presence or absence of the Philadelphia chromosome, influencing treatment strategies and guiding the use of targeted therapies (Dalle et al). Techniques for MRD assessment include multiparameter flow cytometry (MFC), quantitative polymerase chain reaction (PCR) for immunoglobulin/T-cell receptor rearrangements and gene fusions like BCR-ABL1, and next-generation sequencing (NGS), each offering different sensitivities and specificities.[8,9] The timing of MRD evaluation is critical, typically performed at the end of induction therapy, during consolidation, and at various points throughout maintenance therapy to predict relapse and guide ongoing treatment decisions.[10] Despite these advances, gaps remain in the global understanding of MRD, particularly in settings like Bangladesh where data are sparse. This lack of local data hampers the ability to fully integrate MRD assessments into clinical practice, which is crucial for tailoring treatment strategies to individual risk profiles. This study aims to address these gaps by evaluating the incidence and prognostic impact of MRD in ALL patients at a tertiary care hospital in Bangladesh. By correlating MRD status with patient outcomes, this research will contribute valuable insights into the optimization of treatment regimens and the strategic management of patients at high risk of relapse. Ultimately, understanding MRD dynamics within this specific population could lead to improved survival outcomes and a reduction in treatment-related morbidity, aligning with global best practices as recommended by leading oncology societies.[11,12]

This study aims to address these gaps by evaluating the incidence and prognostic impact of MRD in ALL patients at a tertiary care hospital in Bangladesh. By correlating MRD status with patient outcomes, this research will contribute valuable insights into the optimization of treatment regimens and the strategic management of patients at high risk of relapse. Ultimately, understanding MRD dynamics within this specific population could lead to improved survival outcomes and a reduction in treatment-related morbidity, aligning with global best practices as recommended by leading oncology societies.

METHODS

The observational cross-sectional study was conducted in the department of haematology at Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, from August 2021 to July 2022. The study included 22 newly diagnosed ALL patients who received induction chemotherapy under the standard Berlin-Frankfurt-Münster protocol. Patients aged 10 years and above, both male and female, were enrolled following a purposive sampling method, excluding relapsed and transformed cases of ALL and patients younger than 10 years. Measurable residual disease (MRD) was assessed between day 28 to 35 post-chemotherapy using multiparameter flow cytometry (MFC), conducted with a 3-laser 10-color BD FACS Lyric analyzer. For patients with BCR-ABL positive ALL, quantitative PCR was advised in addition to MFC. Ethical approval for the study was obtained from the Institutional Review Board (IRB) of BSMMU. Informed consent was acquired from all participants, ensuring the right to withdraw from the study at any time without any consequence.

Statistical analysis

Data confidentiality was strictly maintained, and statistical analysis was performed using IBM statistical package for the social sciences (SPSS) statistics for Windows, version 22.0. Quantitative variables were presented as mean±standard deviation, and categorical variables were expressed in percentages. The significance of the differences between MRD positive and negative groups was analyzed using unpaired t-tests and Chi-square tests, where applicable, with a p value of less than 0.05 considered statistically significant.

RESULTS

In this study of 22 ALL patients treated with induction chemotherapy, 7 patients (31.82%) were found to be MRD positive, indicating the presence of residual leukemic cells. The remaining 15 patients (68.18%) achieved MRD negative status, showing no detectable leukemic cells (Figure 1).

Among the participants, anaemia was the most prevalent clinical sign, observed in 20 patients (90.91%). Other significant findings included lymphadenopathy and splenomegaly, each noted in 4 patients (18.18%). Haemorrhagic manifestations were present in 6 patients (27.27%), and bony tenderness was also noted in 4 patients (18.18%). Less common signs included hepatomegaly and enlarged tonsils, each observed in 1 patient (4.55%) (Figure 2).

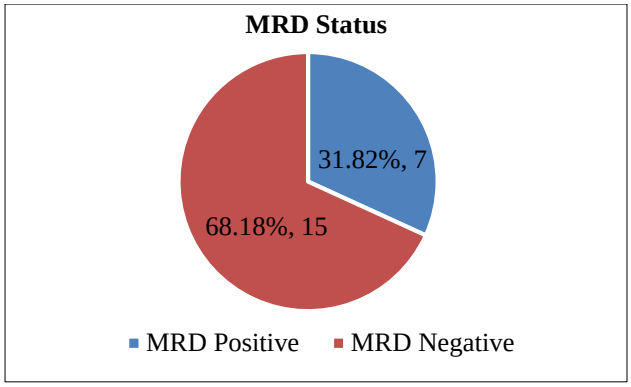

Figure 1: MRD status of study populations after induction chemotherapy (n=22).

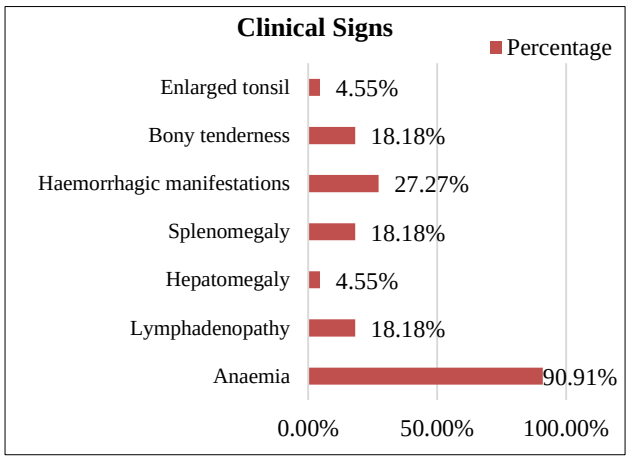

Figure 2: Distribution of clinical signs among the participants (n=22).

Table 1: Demographic and clinical characteristics at diagnosis (n=22).

Variables	MRD positive (n=7)		MRD negative (n=15)		P value
	N	%	N	%	
Age (years)					
10-18	3	42.86	5	33.33	>0.05
19-60	4	57.14	10	66.67	
>60	0	0.00	0	0.00	
Mean±SD	23.14±6.79		26±11.48		>0.05
Sex					
Male	6	85.71	6	40.00	<0.05
Female	1	14.29	9	60.00	

Among MRD positive patients (n=7), 42.86% were aged 10-18 years and 57.14% were aged 19-60 years. In the MRD negative group (n=15), the distribution was 33.33% for ages 10-18 and 66.67% for ages 19-60. No patients over 60 were reported in either group. The mean ages were 23.14±6.79 years for the MRD positive group and 26±11.48 for the MRD negative group, with no significant age difference observed between the two groups (p>0.05). Regarding gender, 85.71% of MRD positive patients were male compared to only 40.00% of the MRD negative patients, showing a statistically significant difference (p<0.05). In contrast, females comprised 14.29% of the MRD positive group and 60.00% of the MRD negative group (Table 1).

The distribution of hemoglobin levels among patients showed no significant difference in MRD status (p>0.05). In the MRD positive group (n=7), 28.57% had hemoglobin levels below 8 gm/dl, 42.86% between 8-10 gm/dl, and 28.57% above 10 gm/dl. Similarly, the MRD negative group (n=15) showed 20.00% below 8 gm/dl, 53.33% between 8-10 gm/dl, and 26.67% above 10 gm/dl. Total leukocyte counts across different ranges also showed no statistically significant association with MRD status (p>0.05). In the MRD positive group, 28.57% had counts below 4×10^9/l and 11-30×10^9/l each, and 14.29% had counts between 4-11×10^9/l. The MRD negative group displayed 13.33% below 4×10^9/l, 33.33% between 4-11×10^9/l, and 6.67% between 11-30×10^9/l. Platelet counts were similarly distributed with no significant differences linked to MRD status (p>0.05). In the MRD positive patients, 28.57% had counts below 25×10^9/l, and 57.14% had counts above 100×10^9/l. The MRD negative group showed 60.00% below 25×10^9/l and only 6.67% above 100×10^9/l.

Percentage of blasts in PBF and bone marrow blast percentage showed no significant variance with MRD status. Nearly all patients in both groups showed a blast percentage between 20-90% in both peripheral blood and bone marrow. Significant differences were observed in serum creatinine and uric acid levels between the MRD positive and negative groups. MRD positive patients had higher mean serum creatinine (1.23±0.31 mg/dl) and uric acid (7.09±1.62 mg/dl) compared to MRD negative patients (0.77±0.24 mg/dl for creatinine and 4.95±1.83 mg/dl for uric acid), with p values of <0.001 and <0.05, respectively. Serum bilirubin levels showed no significant difference between the groups (p>0.05) (Table 2).

In the study population, the distribution of aberrant immunophenotype markers at diagnosis demonstrated no statistically significant association with MRD status, with p-values exceeding 0.05 for all markers. Specifically, CD7 was present in only one MRD negative patient (6.67%), indicating its minimal occurrence. CD13 appeared in 14.29% of MRD positive patients and 40.00% of MRD negative patients. No MRD positive patients exhibited CD15 or CD66c, though these markers were found in 13.33% and 6.67% of MRD negative patients, respectively. CD33 showed a higher presence in MRD positive patients at 28.57%, compared to 13.33% in MRD negative patients. CD117 was detected in one MRD positive patient (14.29%) and was absent in MRD negative patients. Notably, a substantial proportion of both groups - 71.43% of MRD positive and 46.67% of MRD negative patients - did not exhibit any aberrant markers (Table 3).

Table 2: Distribution of laboratory findings at diagnosis and MRD status (n=22).

Variables	MRD positive (n=7)		MRD negative (n=15)		P value
	N	%	N	%	
Haemoglobin (gm/dl)					
<8	2	28.57	3	20.00	>0.05
8-10	3	42.86	8	53.33	
≥10	2	28.57	4	26.67	
Total count (×10^9/l)					
<4	2	28.57	2	13.33	>0.05
4-11	1	14.29	5	33.33	
11-30	2	28.57	1	6.67	
30-100	2	28.57	6	40.00	
≥100	0	0.00	1	6.67	
Total platelet count (×10^9/l)					
<25	2	28.57	9	60.00	>0.05
25-50	1	14.29	2	13.33	
50-100	0	0.00	3	20.00	
≥100	4	57.14	1	6.67	
Blasts in PBF (%)					
<20	1	14.29	2	13.33	>0.05
20-90	6	85.71	13	86.67	
Bone marrow blast (%)					
<20	0	0.00	0	0.00	>0.05
20-90	7	100.00	15	100.00	
>90	0	0.00	0	0.00	
Biochemical parameters (mg/dl)					
Serum creatinine	1.23±0.31		0.77±0.24		<0.001
Serum uric acid	7.09±1.62		4.95±1.83		<0.05
Serum bilirubin	0.67±0.31		0.9±0.38		>0.05

Table 3: Distribution of aberrant immunophenotype markers at diagnosis and MRD status of the study population (n=22).

Aberrant immune-phenotype	MRD positive (n=7)		MRD negative (n=15)		P value
	N	%	N	%	
CD7	0	0.00	1	6.67	>0.05
CD13	1	14.29	6	40.00	>0.05
CD15	0	0.00	2	13.33	>0.05
CD33	2	28.57	2	13.33	>0.05
CD66c	0	0.00	1	6.67	>0.05
CD117	1	14.29	0	0.00	>0.05
No	5	71.43	7	46.67	>0.05

The analysis of BCR-ABL1 status in relation to MRD outcomes in the study population indicated no significant correlation, with a p value greater than 0.05. None of the MRD positive patients (n=7) tested positive for the BCR-ABL1 marker, whereas 26.67% of MRD negative patients (n=4) were BCR-ABL1 positive. Conversely, all MRD positive patients were BCR-ABL1 negative, which aligns with 73.33% of the MRD negative group (n=11) (Table 4).

The study's evaluation of risk stratification in relation to MRD status among 22 patients did not demonstrate a statistically significant correlation, as indicated by p-values exceeding 0.05 across all categories. Within the MRD positive group, 14.29% were classified as low risk, 57.14% as intermediate risk, and 28.57% had unknown risk statuses; no MRD positive patients were in the high-risk category. Conversely, in the MRD negative group, 6.67% were low risk, 33.33% were intermediate, 26.67% were high risk, and 33.33% had unknown risk statuses (Table 5).

Table 4: Distribution of BCR-ABL1 status and MRD status of the study population (n=22).

BCR-ABL1	MRD positive (n=7)		MRD negative (n=15)		P value
	N	%	N	%	
BCR-ABL1 (positive)	0	0.00	4	26.67	>0.05
BCR-ABL1 (negative)	7	100.00	11	73.33	

Table 5: Risk stratification of the study population and MRD status (n=22).

Risk stratify-cation	MRD positive (n=7)		MRD negative (n=15)		P value
	N	%	N	%	
Low	1	14.29	1	6.67	>0.05
Intermediate	4	57.14	5	33.33	
High	0	0.00	4	26.67	
Not known	2	28.57	5	33.33	

Table 6: Bone marrow morphological remission status of study populations and MRD status after induction therapy.

Remission status	MRD positive (n=7)		MRD negative (n=15)	
	N	%	N	%
Complete remission	5	71.43	8	53.33
Complete remission with incomplete count recovery	2	28.57	5	33.33
Complete remission with partial hematologic recovery	0	0.00	2	13.33

The correlation between bone marrow morphological remission status and MRD outcomes following induction therapy reveals varied responses among the study participants. In the MRD positive group (n=7), 71.43%

achieved complete remission and 28.57% reached complete remission with incomplete count recovery, with none in partial hematologic recovery. Conversely, within the MRD negative group (n=15), 53.33% achieved complete remission, 33.33% were in complete remission with incomplete count recovery, and 13.33% reached complete remission with partial hematologic recovery (Table 6).

DISCUSSION

The study of measurable residual disease (MRD) and associated clinical indicators in patients treated for ALL at a tertiary care center provides crucial insights into the dynamics of treatment response and disease monitoring. Our findings revealed that 31.82% of patients were MRD positive after induction chemotherapy, suggesting persistent disease activity despite initial treatment efforts. This MRD positivity rate is somewhat consistent with the observations made by Tembhare et al who reported similar findings in childhood T-cell ALL, emphasizing the prognostic significance of MRD in determining long-term outcomes across various subtypes and patient demographics.[13] The prevalence of clinical signs such as anaemia in 90.91% of our participants points to the aggressive nature of ALL and its profound impact on hematologic function. This observation is indicative of the disease's severity and the body's response to both leukemia and chemotherapy, which often manifests as widespread hematologic suppression.[14] The literature corroborates that anemia is a common complication in leukemia patients, reflecting the high turnover of malignant cells and marrow infiltration that disrupts normal hematopoiesis.[10] In our cohort, the demographic analysis revealed no significant age difference between MRD positive and negative groups, suggesting that age, within the adult range studied, does not independently predict MRD outcomes. This finding aligns with broader oncological research that has produced mixed results regarding age's influence on MRD positivity and overall prognosis in ALL.[10] However, the significant gender disparity observed with a notably higher percentage of males being MRD positive raises questions about potential biological or treatment-related differences between genders. This aspect of MRD positivity is less frequently discussed in literature but echoes the need for further studies to explore how gender might influence treatment outcomes or disease progression in leukemia. The elevated levels of serum creatinine and uric acid in MRD positive patients highlight a potentially higher tumor burden or increased toxicity from treatment. These biochemical markers, often reflective of renal function and cell turnover, can serve as indirect indicators of disease activity or complications arising from the therapy itself.[15-17] The implication is that monitoring these levels could provide additional layers of information for adjusting treatment protocols or for prognostic assessments. Our study also delved into the expression of aberrant immunophenotype markers, notably CD33, which was more prevalent among MRD positive patients. This finding is intriguing as CD33 has been targeted in therapeutic contexts, particularly in myeloid leukemias, with varying success. The presence of such markers could potentially refine risk stratification and treatment customization in ALL, aligning with recent advances in targeted therapies that exploit specific cellular markers for more effective disease control.[18] The absence of the BCR-ABL1 genetic marker in MRD positive patients, contrary to a subset of MRD negative patients who tested positive, suggests a complex interplay between genetic factors and MRD status. The BCR-ABL1 oncogene, associated with Philadelphia chromosome-positive ALL, generally indicates a more aggressive disease course and a different therapeutic approach, typically involving tyrosine kinase inhibitors. The differential presence of this marker across MRD statuses may reflect distinct biological behaviours and treatment responses, underscoring the heterogeneous nature of ALL.[19] Risk stratification did not show a significant correlation with MRD status in our study, challenging the conventional reliance on risk categories to predict MRD outcomes.[9,20] This discrepancy suggests that traditional risk models may need recalibration or augmentation with molecular and genetic data to enhance their predictive accuracy for MRD. The higher rate of complete remission observed among MRD positive patients contrasted with the lower complete remission rate but inclusion of partial hematologic recovery in MRD negative patients adds another layer of complexity to understanding MRD dynamics. This phenomenon could indicate that MRD negativity, even in the presence of less than complete morphological remission, may still portend a favourable prognosis, potentially altering how remission is evaluated and managed in clinical practice. In summary, our findings contribute valuable knowledge to the ongoing discourse on managing and prognosticating ALL, highlighting the multifaceted nature of MRD and its interrelations with clinical, demographic, and biochemical parameters. Future research should continue to dissect these relationships and explore innovative approaches to integrate these variables into a cohesive strategy for personalized medicine in ALL.

Limitations

The study was conducted in a single hospital with a small sample size. So, the results may not represent the whole community.

CONCLUSION

This study reaffirms the critical role of MRD as a prognostic marker in ALL, demonstrating that a significant portion of patients (31.82%) remain MRD positive after induction chemotherapy, indicative of persistent leukemic activity. The presence of MRD positively correlates with higher levels of key biochemical markers, such as serum creatinine and uric acid, suggesting a higher tumor burden or increased treatment toxicity. Additionally, the study highlights demographic nuances, with a notable gender disparity in MRD positivity rates and emphasizes the importance of including comprehensive

immunophenotypic profiling to better stratify risk and tailor treatments. The absence of the BCR-ABL1 marker in MRD positive patients underscores the heterogeneity of the disease and suggests differential pathways in disease progression and response to treatment. Overall, the findings advocate for an integrated approach that combines MRD assessment with detailed clinical and laboratory diagnostics to optimize treatment strategies, improve patient outcomes, and move towards more personalized therapy in ALL management.

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