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Minimal Residual Disease in Pediatric Acute Lymphoblastic Leukemia

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Abstract:

Background and Objective The detection of minimal residual disease at the end of initial therapy in Acute Lymphoblastic Leukemia to assess risk stratification is gaining clinical significance. While minimal residual disease assesses the extent of remission, remission is still defined by bone marrow morphology. The aim of this study is to determine the significance of minimal residual disease.

Methods: In this cross-sectional study conducted at Zheen Oncology Center and Azadi hospital in Duhok, Iraq, data was collected from 46 patients' records. Residual disease was detected by flow cytometry. On day 29 of induction therapy, a bone marrow sample was obtained for morphology and flow cytometry. The presence or absence of MRD was determined by using 8-color flow cytometry.

Results: The median age of the study was 7 years and the male to female ratio was 1.7:1. B-cell lymphoblastic leukemia accounted for 80.43% and T-cell was 19.57%. Bloodounts at diagnosis revealed a mean white blood cell count of 74.32 x 10^9/L, mean hemoglobin level of 8.75, g/dL, and a mean platelet count of 97.84 x 10^9/L. Minimal residual diease positive results were seen in 29 cases; 21 cases were of B-cell leukemia (56.75) and 8 (88.88) cases were T-cell leukemia. Minimal residual disease negative results were achieved in 17 cases; 43.24% were of B-cell origin (p value <0.001), 11.11% were of T-cell origin (p value 0.008). Remission was achieved in 81.08 cases of B-cell (p value 0.103), and 66.66 of T-cell (p value 0.403). 16.21% of B-cell leukemia passed away (p value <0.001) and 33.33% of T-cell leukemia (p value 0.065).

Conclusion: Flow cytometry should be done in addition to bone marrow morphology.

Key words: Acute Lymphoblastic Leukemia, Flow Cytometry, Minimal Residual Disease, Morphology.

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease caused by abnormal proliferation of immature lymphoid cells and has become the most common malignancy in children. Leukemia accounts for about 30% of childhood cancers, with acute lymphoblastic leukemia (ALL) making up nearly 80% of cases.[1-3] Despite recent advancements in treatment, nearly a quarter of patients with standard risk still relapse. [1] Relapse causes morbidity and mortality. Relapse is defined by the presence of extremely low numbers of leukemic cells still present once complete remission is obtained. This phenomenon is called minimal residual disease (MRD). MRD has the ability to detect one malignant cell out of 100,000 normal cells.[2]This means that cancer cells that cannot be seen under the microscope, but can emerge and cause relapse. These cells need more sensitive testing. MRD can be detected by flow cytometry, PCR, or genetic tests. The remaining leukemic cells typically do not cause symptoms; however, they can proliferate at any time and cause relapse. Minimal residual disease (MRD) has gained a key role in the management of childhood and adult ALL. It is used as a high prognostic factor. Negative results during early stages of treatment may decrease unnecessary intense regimens and therefore decrease complications of intense treatment. It may indicate patients who are more probable to be cured with reduced intensity regimens. However, positive MRD levels do not necessarily indicate failure of induction. The degree of positivity is also important. A patient with a high level of MRD is at greater risk than a positive result with a relatively low level of MRD. Currently, patients with a normal bone marrow morphology and negative MRD, are said to be in complete remission. Sometimes, there is discrepancies between the MRD results and bone marrow morphology. Morphologic remission is the presence of less than 5% blast cells in the bone marrow. Immunophenotypic remission is achieving MRD negativity after therapy. It is difficult to say if one method is preferred over the other. In our locality, remission is still defined by morphology, despite the difficulties hematologists face in differentiating between malignant lymphoblasts and hematogones (non-malignant regenerating cells).[3,4] This study aims to determine the significance of MRD in assessing remission in children with discordant results between morphology and flow cytometry at the end of induction therapy.

Patients and methods:

This study was conducted at the Azadi hospital and Zheen Oncology Center in Duhok. Data from 46 patients' records were collected from August 2019 through December 2021. All patients were below the age of 16 years. Patients older than 16 years, those diagnosed with myeloid leukemia, and those whose records were unavailable were excluded. The KHCMS approved this study and consent was obtained from all parents/guardians. The original diagnosis of leukemia was made by peripheral blood morphology, bone marrow examination and/or flow cytometry. Diagnosis was made by the presence of ≥20% lymphoblasts by either peripheral blood or bone marrow aspirate. The diagnosis was confirmed by flow cytometry. All patients were treated according to the UKALL protocol, except one patient who was treated by the infant protocol. On day 29 of induction therapy, a bone marrow sample was obtained for morphology and flow cytometry. Morphology was assessed independently in the diagnostic lab. The presence or absence of MRD was determined by using 8-color flow cytometry at a different lab. The detection of MRD should have the following features: (a) sensitivity of at least 10^{-4} (one

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malignant cell within 10,000 normal cells); (b) specificity, to distinguish between malignant and normal cells; (c) be quantifiable within a large dynamic range; (d) stability over-time of leukemia-specific markers, to prevent false-negative results, particularly in long-term studies; (e) reproducibility between laboratories; (f) careful standardization and quality control checks; (g) rapid availability of results. [1'5'6] All samples were tested for TdT, CD10, and CD19. [7-9] It should be known that leukemic blasts lose CD99 and TdT during induction therapy. [7] Flow cytometry was performed using a Becton Dickinson FACScan with LYSYS II software. Each sample was acquired twice with each sample including at least 100,000 cells. Morphologic assessments of bone marrow aspirates and MRD were both performed at local hospitals. Patients were divided into 3 groups by morphologic assessment: Category 1, C1 (<5% blasts), Category 2, C2 (5-20% blasts), and Category 3, C3 (>20% blasts). Demographic data were summarized; means and ranges were used for continuous data, and percentages and frequency were used for categorical variables Ethics approval has been given by the Ministry of Health, Duhok, Iraq.

Results:

A total of 46 patients with ALL were evaluated for MRD in this study. Their ages ranged from 0.5 to 15 years, with a median of 6.5 years. The study included 29 males and 17 females, with a ratio of 1.7:1. Blood counts revealed a mean hemoglobin of 8.6 g/dL (range 4.8-11.9), a mean leucocyte count of 74.3 x 10^9/L (range 1.7-469), and a mean platelet count of 96.6 x 10^9/L (range 11-443). Furthermore, morphology and immunophenotype analysis revealed 37 cases (80.9%) of B-cell lineage and 9 cases (19.6%) of T-cell lineage. The majority of the B-cell cases were of the common B-ALL type (94.6%), with the rest of the cases being Pro-BALL type. Cortical T-ALL was the predominant type among the T-cell ALL, followed by Early T-cell ALL (54.6% and 44.4%, respectively). These demographic characteristics are shown in Table (1).

Table (1): Demography and outcome of patients

	MRD +ve	MRD –ve	Remission	Death	Relapse
<7 yrs	13	10	18	5	
≥7 yrs	16	7	16	6	1
p value	0.727	0.331	0.154	0.727	0.324
Male	18	11	19	8	1
Female	11	7	15	3	
p value	0.008	0.002	0.035	0.077	0.324
B-cell	21	16	28	8	1
T-cell	8	1	6	3	
p value	<0.001	<0.001	<0.001	0.096	0.322

After being diagnosed, the patients underwent induction therapy for 28 days, after which bone marrow aspirates were tested for MRD by morphology and flow cytometry on day 29. By morphology, 34 cases were in complete remission; by flow only 17 cases. 15 cases were found to have residual blasts >5%. 18 cases had discrepancies between morphology and flow results. Twenty-one cases were positive for MRD by flow results, which accounts for 56.8% of B-cell cases. Eight of the nine cases of T-ALL, tested positive for MRD (88.9%) by flow cytometry. Of 37 B-ALL cases, 27 cases were in C1 category (morphologically in remission), and 9 cases were in C2 category, and one case in the C3 category. 13 of the cases in C1 category were MRD +ve and 14 were MRD –ve by flow cytometry. In the C2 category, only 2 out of the 9 cases had discordant results between morphology and flow results (22.2%). There was 100%

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correlation between morphology and flow results in the C3 category. In T-ALL, 4 cases achieved morphological remission (C1), and 3 were in the C2 category and 2 in the C3 category. 3 of the cases in C1 category were MRD +ve and 1 was MRD –ve by flow cytometry. There was 100% correlation between morphology and flow results in the C2 and C3 category. In the majority of the patients (78.3%), the morphology and flow results were consistent with one another. 21 patients with C1 morphology had positive MRD (46.7%), and 2 patients with C2 morphology had negative MRD (16.7%). High flow results indicating positive MRD in patients with C1 morphology was seen more frequently, and there was no significant difference between the two immunophenotypes. 3 patients with C1 morphology/MRD+ve passed away (18.8%), 18 patients achieved remission in the last follow-up. Those with C2/C3 morphology but negative MRD were much less common (8.7%). This suggests that MRD is a significant prognostic factor.[3-7] Assessment of morphology and flow cytometry are shown in Table (2).

Table (2): Assessment of morphology and flow cytometry on day 29

	C1/Blasts <5%	C2/Blasts 5-20%	C3/Blasts >20%
MRD +ve	16	10	3
MRD - ve	15	2	0
p value	0.154	0.003	0.073

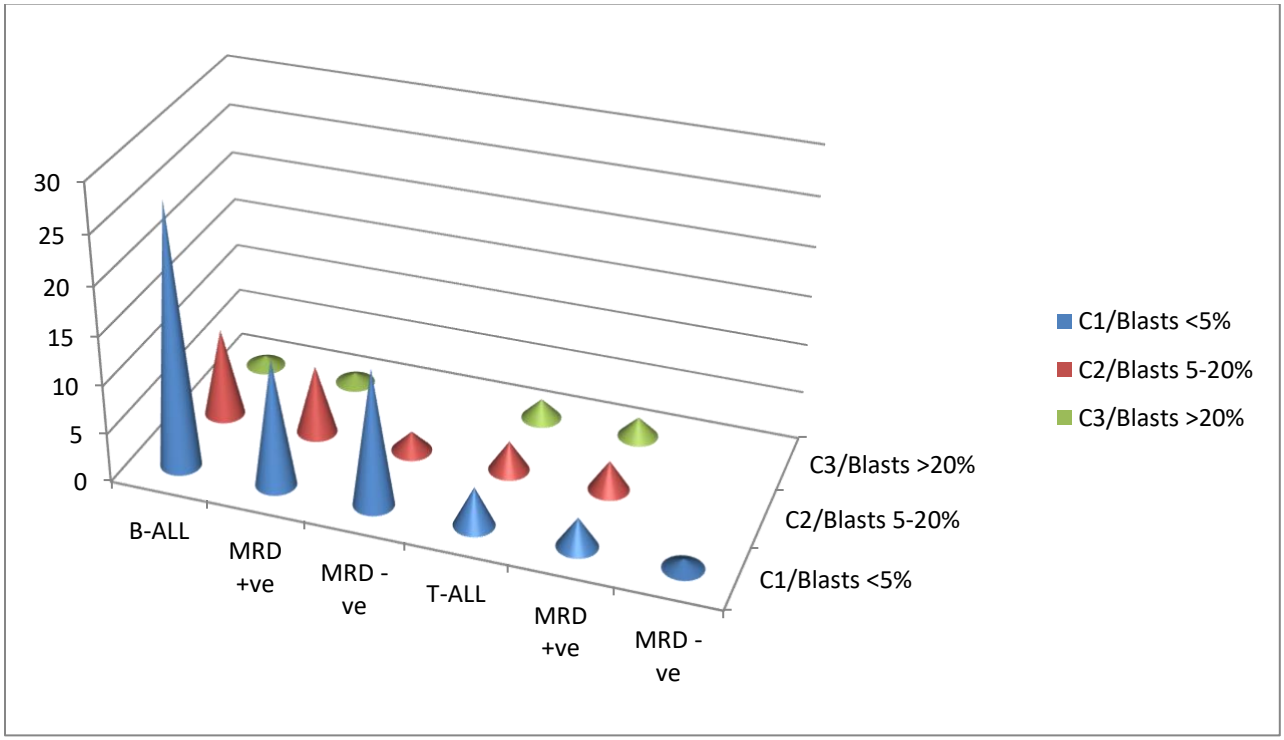

Figure (1): Morphology vs. MRD and immunophenotype

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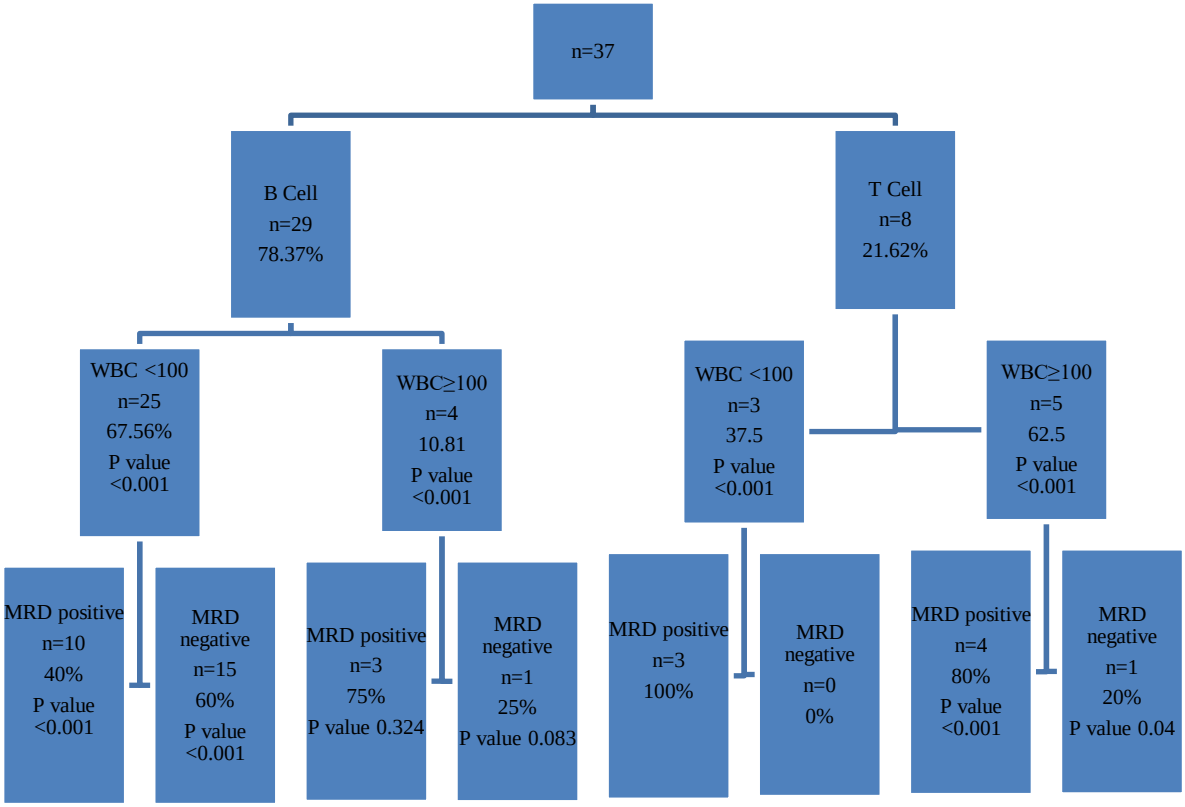

Figure (2): Relationship between Immunophenotype, WBC count, and MRD

Discussion:

Bone marrow morphology plays a crucial role in the diagnosis and follow-up of acute leukemia. However, assessment of remission by morphology alone is limited. It is well-known that morphologic assessment of the bone marrow after chemotherapy can be troublesome as it may be very difficult to distinguish malignant lymphocytes from non-malignant cells (hematogones).[3,10-12] This problem can be overcome by flow cytometry, which is able to differentiate between hematogones and malignant lymphoblasts accurately. Also, it may be difficult to count malignant lymphocytes if they are present in small numbers or if they are scattered. Regardless, bone marrow morphology remains an important procedure to assess remission. In recent years, MRD measured by flow cytometry has helped in defining remission. It allows the clinician to understand the depth of remission. This new method can detect even the smallest number of malignant cells.[11] Our ability to determine the clinical significance of those with C2/C3 morphology but negative MRD is poor due to the small number of patients in this category. This study included 46 patients with ALL with ages between 6 months and 15 years. We established that morphology remains an accurate method to assess MRD after the completion of induction therapy in children with ALL. Our study concluded that morphologic and flow cytometric assessment of remission was concordant in the vast majority of cases. We determined the clinical

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significance of MRD detection and found that conventional morphology should not be replaced by MRD in assessing remission. [3] MRD interprets the depth of remission.[3,8] The use of both morphology and MRD together is needed for assessment of complete remission and correlate with patients' outcomes. Our study was comparable with few other studies done in India, London that found that neither method can replace the other.[8]Our study showed that the presence of <5% blasts in the bone marrow did not necessarily indicate complete remission as a positive MRD was found in 16 patients.[8] Similarly, the presence of >5% blasts did not indicate relapse as these cells might have been non-malignant B-cell precursors. Also, MRD positive cases does not always correlate with long term survival.[5,13,14]Our study found that the outcome of patients with C1/MRD≥5% was not statistically different from those concordantly not in remission. [3,15,16] Some studies suggested that clinicians should fully rely on MRD results and omit bone marrow morphology.[3,6,17-19] The main concern is whether to use MRD in selecting or changing therapy protocols. Our study showed that we cannot solely depend on MRD alone.[5,14,20-23] Unfortunately, the number of patients in our study was limited as MRD is a relatively new concept in our locality. It is possible that in the near future more advances in MRD and cytogenetic analysis can alter our current therapy regimens; and possibly be used as more accurate tests than morphology to determine remission. The clinical value of MRD should be investigated by a larger trial.

Conclusion:

We conclude that MRD should not replace morphology but rather be used in conjunction, as MRD can give us a more accurate representation of remission status. MRD by flow cytometry is a simple and sensitive procedure to predict the outcome of patients with ALL.

Conflict of interest

The author reports no conflicts of interest.

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