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THE SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKAEMIA: A SINGLE CENTRE STUDY

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ABSTRACT:

In Acute lymphoblastic Leukemia (ALL) assessment of molecular response to treatment, assessing minimal residual disease (MRD) is a major independent predictor of treatment outcome. Consequently, MRD is implemented in all ALL-treatment protocols to fill up or to redefine stratification of multifactorial risk with optional intensity of customized treatment.
Aim: to specify the significance of MRD in the assessment of remission in children with ALL with results discordant between morphology and flow cytometry at the end of induction phase of therapy.
Materials and Methods: A descriptive cross-sectional study was conducted at Jin Oncology Center from March 2019 through November 2023. Data were taken out of the records of 58 patients who had ALL less than 16 years old. All patients were less than 16 years old and treated by ukall. They were diagnosed using peripheral blood morphology, bone marrow study and/or flow cytometry when lymphoblasts in peripheral blood or bone marrow aspirate are ≥20% and was confirmed by flow cytometry. On 29th day of induction therapy, bone marrow was examined for morphology and flow cytometry. The presence or absence of MRD was determined, and CD19, CD10 and tdt were tested. By morphologic assessment they were divided patients into: Category 1, C1 (<5% blasts), Category 2, C2 (5-20% blasts), and Category 3, C3 (>20% blasts). Statistical analysis was made using SPSS version 25. P value of less than 0.05 was considered significant.
Results: The study involved 58 patients who had ALL. with a median age of 6.5 years, male to females ratio of 1.76:1, mean platelet count of 96.6 x 10^9/L ,mean hemoglobin of 8.6 g/dL, mean leucocyte count of 74.3 x 10^9/L), 48 cases (82.7%) of B-cell lineage and 10 cases (17.3%) of T-cell lineage, 94.6% of the B-cell cases were of the common B-ALL and the rest Pro-BALL type, 54.6% of the T-cell ALL was cortical T-ALL and 44.4% Early T-cell ALL. They were tested for MRD by morphology and flow cytometry on day 29. By morphology, 46 patients had remission but by flow only 24 cases. Seventeen cases had residual blasts >5%. In 19 cases there was a discrepancy between the results of morphology and flow. Twenty-five cases (52.08% of B-cell cases) were positive for MRD by flow results. Eight of the ten cases of T-ALL (80%), were positive for MRD by flow cytometry. Among 48 cases of B-ALL, 36 were in C1 category (morphologically in remission), 11 cases were in C2 category and one case in the C3 category. Of cases in C1 category, 17 were MRD +ve and 19 were MRD –ve by flow cytometry. In the C2 category, only 2 out of the 11 cases (18.18%) had discordant results between morphology and flow results. The correlation between morphology and flow results was 100% in the C3 category.
Conclusion: MRD should not be the surrogate of morphology but to be used in conjunction in order to give us a more accurate representation of status of remission.
KEYWORDS: Leukemia, minimal residual, flowcytometry, remission.

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is considered the most common malignancy of paediatric age. Its incidence in the United States is 1.4 cases per 100,000 population[1] . The outcome of treatment has significantly been better and the evolving stratification of risk, allowing for more customized treatments. in the past 40 years due to intensification of treatment, central nervous system (CNS) prophylaxis and the developing stratification of risk thus helping more customization of treatment. Various biological and clinical variables influence the treatment outcome. The main variables included white blood cell count, age, blast genotype, the involvement of CNS at diagnosis and the treatment's initial response to which is measured by assessing the regression of the disease[2,3].

Detecting malignant cells which are still present in the body (residual disease) while receiving treatment of acute leukemia and after that is the best means by which we can monitor the response to treatment and to predict its relapse. In general, a stronger response achievement leads to a better prognosis. Although complete remission (CR) is achieved by most of the patients according to both clinical and morphological criteria, a lot of patients may relapse. It is evident that not all malignant cells are necessarily damaged in the patients who have CR, and the residual disease level is significantly related to the risk of relapse and outcomes of survival[4-7] .

Over the last three decades, many studies were conducted on the clinical significance of minimal residual disease (MRD) and its detection methodologies. These studies concluded that MRD is the most reliable independent factor that predicts the relapse and outcome of survival [8-10]. Testing of MRD is now included in the management of some ALL patients whose treatments are modified according to the status of MRD. We can also use MRD as an alternative endpoint to hasten the testing and the process of approval of a novel treatment or a new treatment product [11,12].

In ALL, assessment of molecular response to treatment, assessing MRD is a considered a major independent predictor of treatment outcome, as proven by various studies [13,14]. As a result MRD is included in all ALL-treatment protocols aiming to supplement or to fill up stratification of multifactorial risk with optional intensity of customized treatment. Detection of leukemic cells below the limit of classical cytomorphology is workable either by changes of the immune phenotype which are disease-specific or by unique genetic features. There has been development of several contending and completing MRD methods with preference application according to clinical protocols[15] .

In Duhok Governorate and all Kurdistan Region, remission of ALL is still defined depending on morphology in spite of the difficulties enfaced by hematologists to differentiate malignant lymphoblasts from hematogones (non-malignant regenerating cells) [16,17].

This study aims to specify the significance of MRD in the assessing the remission in ALL children who have results discordant between morphology and flow cytometry at the end of induction phase of therapy.

2. MATERIALS AND METHODS:

This descriptive cross-sectional study was conducted at Jin Oncology Center in Duhok, Kurdistan Region, Iraq from March 2019 through November 2023. Data from the records of 58 patients were collected. These data included age, gender, platelet count, haemoglobin, leucocyte count , B cell and T cell lineage and their subtypes, residual blasts and C1, C2 and C3 categories. All patients were less than 16 years old. We excluded from the study those who were older than 16 years, those having myeloid leukemia and those with unavailable records. The ethical committee at the directorate pf health of Duhok approved this study and a written consent was obtained from all parents/guardians.

The leukaemia cases were originally diagnosed using peripheral blood morphology, bone marrow study and/or flow cytometry. Diagnosis was considered when there is ≥20% lymphoblasts in peripheral blood or bone marrow aspirate. The researchers confirmed the diagnosis by flow cytometry. United Kingdom Acute Lymphocytic Leukaemia (UKALL) protocol was used to treat all the patients and included chemotherapy (Vincristine, Aspraginase , anthracycline , cyclophosphamide and Intrathecal chemotherapy) protocols, supportive care measures, and guidelines for monitoring patients' progress. while one patient was treated by the infant protocol. On 29th day of induction therapy, the researchers obtained a bone marrow sample and examined it for morphology and flow cytometry. This is a medical procedure used to collect a sample of bone marrow for examination to diagnose of Lukemia and other hematological disorders. Independent assessment of morphology was done in the diagnostic lab.

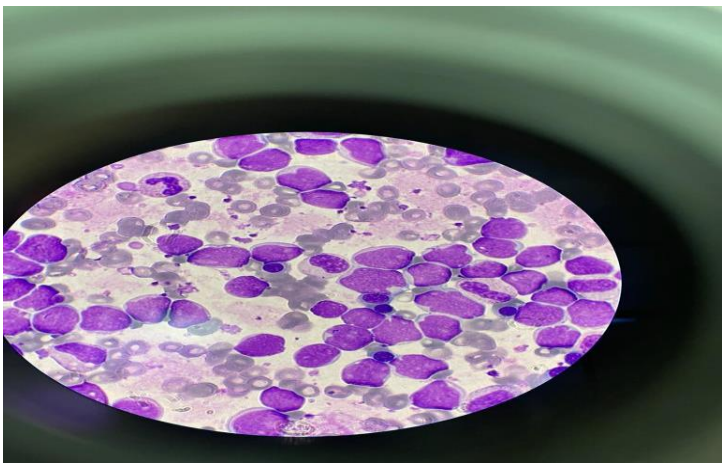

Figure 1: Bone marrow slide showing blast cell of ALL

The presence or absence of MRD was determined by using 8-color flow cytometry at Heevi Hospital lab. In 8-color flow cytometry, researchers use up to eight different fluorochromes conjugated to antibodies targeting specific cell surface markers. This allows for the simultaneous detection of a more extensive panel of marker lab. The detection of MRD is defined by the presence of: (a) sensitivity of at least 10^{-4} (one malignant cell within 10,000 normal cells); (b) specificity, to distinguish between malignant and normal cells; (c) be measurable within a big dynamic range; (d) over-time stability of leukemia-specific markers, to avoid getting false-negative results, especially in long-term studies; (e) ability to duplicated between laboratories; (f) careful comparison to standards and quality control checks; (g) rapid accessibility of the results [18-20].

The researchers tested all samples for Cluster of Differentiation (CD)19, CD10 and Terminal Deoxynucleotidyl

Transferase (TdT) [21,22]. It is well known that the leukemic blasts lose TdT and CD99 during induction therapy [21]. Flow cytometry was performed using a Becton Dickinson FACScan with LYSYS II software. The researchers acquired each sample twice with each sample including at least 100,000 cells. Morphologic assessments of bone marrow aspirates and MRD were both performed at local hospitals. By morphologic assessment, the researchers divided patients into 3 groups: Category 1, C1 (<5% blasts), Category 2, C2 (5-20% blasts), and Category 3, C3 (>20% blasts).

Statistical analysis was made using SPSS version 25. Demographic data were summarized; means and ranges were used for continuous data, and percentages and frequency were used for categorical variables. P value of less than 0.05 was considered significant.

3. RESULTS

The study included 58 patients with ALL. Their ages were between 0.5 and 15 years, with a median of 6.5 years. The study included 37 males and 21 females, with a ratio of 1.76:1. Blood counts revealed mean platelet count of 96.6 x 10^9/L (range 11-443), a mean hemoglobin of 8.6 g/dL (range 4.8-11.9) and a mean leucocyte count of 74.3 x 10^9/L (range 1.7-469), and a Furthermore, morphology and immunophenotype analysis revealed 48 cases (82.7%) of B-cell lineage and 10 cases (17.3%) 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. The pre-dominant type among the T-cell ALL was cortical T-ALL followed by Early T-cell ALL (54.6% and 44.4%, respectively). The demographic characteristics of patients are shown in Table (1).

Table 1: Demography and outcome of patients

	MRD +ve	MRD –ve	Remission	Death	Relapse
<7 yrs	15	15	25	5	
≥7 yrs	18	10	22	5	1
P value	0.727	0.331	0.154	0.727	0.324
Male	22	15	27	10	1
Female	11	10	17	4	
P value	0.008	0.002	0.035	0.077	0.324
B-cell	25	23	39	8	1
T-cell	8	2	7	3	
P value	<0.001	<0.001	<0.001	0.096	0.322

Soon after the diagnosis, induction therapy for 28 days was started then after this time bone marrow aspirates were done for the patients and were tested for MRD by morphology and flow cytometry on day 29. By morphology, 46 patients had remission but by flow only 24 cases. Seventeen cases had residual blasts >5%. In 19 cases, there was a discrepancy between the results of morphology and flow. Twenty-five cases (52.08% of B-cell cases) were positive for MRD by flow results. Eight of the ten cases of T-ALL(80%), were positive for MRD by flow cytometry. Among 48 cases of B-ALL , 36 were in C1 category (morphologically in remission), 11 cases were in C2 category and one case in the C3 category. Of cases in C1 category, 17 were MRD +ve and 19 were MRD –ve by flow cytometry. In the C2 category, only 2 out of the 11 cases (18.18%) had discordant results between morphology and flow results. The correlation between morphology and flow results was 100% in the C3 category. In T-ALL, 5 cases achieved morphological remission (C1), and 3 were in the C2 category and 2 in the C3 category. 4 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 most of the patients (78.3%), the morphology and flow results were consistent with one another. Twenty one patients (51.21%) with C1 morphology had positive MRD, and 2 patients (14.28%) with C2 morphology had negative MRD. 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. Three patients (14.28%) with C1 morphology/MRD+ve passed away while 18 patients achieved remission in the last follow-up. Those with C2/C3 morphology but negative MRD were much less common (11.76%). This suggests that MRD is a significant prognostic factor[16,17,20,21].

Table 2:. Assessment of morphology and flow cytometry on Day 29

	C1/Blasts <5%	C2/Blasts 5-20%	C3/Blasts >20%
MRD +ve	21	12	3
MRD -ve	20	2	0
P Value	0.154	0.003	0.073

4. DISCUSSION

Morphology study of the bone marrow has a significant role in diagnosing and following-up ALL patients. But the bone marrow assessment after receiving chemotherapy can be problematic because it may be hard to distinguish malignant lymphocytes from non-malignant cells (hematogones)[17, 23,24,26]. Flow cytometry can help us overcome this problem since it can accurately distinguish hematogones from malignant lymphoblasts. Besides, it is not easy to count malignant lymphocytes when they exist in small numbers or if they are scattered. However, bone marrow morphology remains an essential procedure for assessing remission. In recent years, measuring MRD by flow cytometry has helped in defining remission. It helps the clinician to figure out the depth of remission. Even the smallest number of malignant cells can be detected by this new method[25].

Since there are a few patients with C2/C3 morphology but negative MRD, we were poorly able to determine the clinical significance of those in this category.

Our study included 58 patients with ALL with ages between 6 months and 14.8 years. We found that morphology is still an accurate method for assessment of MRD after completing the induction therapy. We concluded that in most of the cases of ALL, there was concordance between flow cytometric and morphologic assessment of remission. We did recognise the clinical significance of MRD detection and confirmed that conventional morphology should not be replaced by MRD in assessing remission [17]. MRD can interpret the depth of remission[17,26]. Using both morphology and MRD together is needed for assessing the complete remission and correlating with outcomes of patients. Our study was in line with a few other studies done in India and London that found that neither method can replace the other [26].

This study showed that finding less than 5% blasts in the bone marrow did not confirm that remission is complete because a positive MRD was found in 16 patients. Similarly, finding more than 5% blasts did not confirm the relapse as these cells might have been non-malignant B-cell precursors. Also, MRD positive cases are not always associated with long term survival[19,27,28].

Unfortunately, we had a limited number of patients in this study since MRD is a relatively new concept in our locality.

CONCLUSION

We conclude that MRD should not be the surrogate of morphology but to be used in conjunction in order to can give us a more accurate representation of status of remission. We recommend larger sample trials in the future to investigate the clinical value of MRD.

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