

## RESEARCH ARTICLE

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# Minimal Residual Disease in Adult Acute Lymphoblastic Leukemia: Egyptian Experience

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### Abstract

**Background:** Acute lymphoblastic leukemia (ALL) is a clonal disease that affects early lymphoid progenitors in the bone marrow. Minimal residual disease (MRD) is assessed by different methods to monitor disease kinetics after treatment. **Aim:** to Assess MRD post-induction, at 6 and 12 months after intensive chemotherapy in adult patients with ALL. **Patients and Methods:** Seventy adult newly diagnosed acute lymphoblastic leukaemia patients were enrolled between July 2018 and July 2019 at the Clinical Hematology Unit, Ain Shams University hospitals, Egypt. MRD was assessed on the bone marrow samples using multi-parameter four color flow cytometry with 0.01% cut-off; below which cases are deemed MRD negative. **Results:** After the end of induction period, 13 out of 46 patients (28%) had positive MRD. However, MRD positivity is demonstrable in 14/32(43.8%), and 10/28(35.7%) patients at 6 and 12 months; respectively. MRD positivity was significantly associated with older age group (more than 39 years) and high NCCN risk stratum with p-values <0.05. Moreover, most of MRD positive patients at 12 months of therapy were of T-ALL immunophenotype (P value 0.002). Patients with complete remission and negative MRD exhibited significantly higher overall survival when compared to patients having MRD positivity (P value 0.027). **Conclusion:** MRD is a powerful predictor of outcome in ALL and its positivity at different time points is associated with poor prognostic factors as well as survival outcomes.

**Keywords:** ALL- MRD- adults- flowcytometry

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### Introduction

Acute lymphoblastic leukaemia (ALL) is a type of haematologic cancer where lymphoid blasts exhibit arrested maturation and differentiation. This in turn results into the accumulation of early lymphoid progenitors in the bone marrow, peripheral blood as well as extramedullary sites; leading to perturbed haematopoiesis and extramedullary infiltrations (Della Starza et al., 2019).

Minimal residual disease (MRD) assessment in ALL patients has been routinely adopted in paediatric ALL. Recently, it has shown more extensive application in adult ALL. This allows better characterization of ALL patients and segregation according to their MRD status into risk groups allowing for the individualization of treatment decisions. Low risk group exhibits MRD negativity and may benefit from treatment reduction limiting toxicity from chemotherapeutic regimens, whereas high risk group with positive MRD may need treatment intensification. Moreover, MRD diagnostics are used in the assessment of efficacy of novel agents like monoclonal antibodies and CAR T-cell therapy (van Dongen et al., 2015).

Minimal residual disease measurement in ALL patients has been performed by either multi-parameter

colour flowcytometry or polymerase chain reaction. The first method relies upon the identification of the original leukaemia associated immunophenotype (LAIP), while the second one performs amplification of immunoglobulin and T-cell receptor gene rearrangements or other aberrant genes associated with the disease (Kruse et al.,2020). In this study, we aim at the evaluation of MRD status in adult ALL patients following induction treatment, at 6 and 12 months after treatment. Furthermore, we aim at the assessment of the relationship of MRD status from one side to other prognostic variables and therapy outcomes from the other side.

### Materials and Methods

#### *Patients and Methods*

Seventy newly diagnosed adult acute lymphoblastic leukemia (ALL) patients were recruited in the study held between July 2018 and July 2019 from Clinical Hematology Unit, Ain Shams University hospitals, Egypt. patients gave informed written consents for the withdrawal and the use of samples for research purposes. The study conformed to the stipulations of declaration of Helsinki of 1975, and its amendments in 2008. All patients were

followed up for one year.

Patients included in the study were newly diagnosed de-novo adult cases of acute lymphoblastic leukaemia that were deemed fit to intensive chemotherapy. Secondary cases on top of chronic myeloid leukaemia, and those who were too frail to receive chemotherapy of curative intent were excluded.

Patients were risk stratified according to the National Comprehensive Cancer Network (NCCN) risk stratification criteria of 2015 into standard risk and high risk (Alvarnas et al., 2015). High risk patients exhibited high risk features including Philadelphia chromosome positivity, complex karyotype, age >35 years, the presence of extramedullary disease, high WBC count at diagnosis ( $>30 \times 10^9/L$  for B-ALL;  $>100 \times 10^9/L$  for T-ALL), or 11q23 positivity. Patients who did not show any of the high-risk criteria were considered standard risk.

#### *Treatment protocols*

All patients received hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone (Hyper-CVAD) cycles alternating with high dose methotrexate and cytarabine cycles that are detailed elsewhere (Kantarjian et al., 2004). Patients with Philadelphia chromosome positive ALL received imatinib 400 mg PO OD in conjunction with the chemotherapy cycles. After the achievement of complete remission, repetition of the cycles was done till a maximum of 4 cycles. After that, maintenance treatment using prednisone, vincristine, methotrexate and 6-mercaptopurine (POMP) was given as mentioned elsewhere (Kantarjian et al., 2004). Patients were followed up for one year.

Refractory/ relapsed cases were given Fludarabine, cytarabine, granulocyte colony stimulating factor and idarubicin (FLAG-IDA) protocol described elsewhere (Specchia et al., 2005).

#### *Response criteria*

Response was assessed according to the NCCN guidelines published in 2015. Complete remission (CR) is considered when bone marrow blasts  $<5\%$ , absolute neutrophil count  $>1 \times 10^9/L$ , and platelet count  $>100 \times 10^9/L$ , with the disappearance of any extramedullary disease. This should persist for at least four weeks. Failure to achieve these targets define chemoresistance, whereas reemergence of bone marrow blasts or de-novo extramedullary disease after the initial response defines relapse (Alvarnas et al., 2015). Minimal residual disease is considered negative at a cut-off 0.01% (Campana, 2010).

#### *Minimal residual disease evaluation using multiparameter four colour flowcytometry*

Bone marrow samples were collected from patients on day 28 post-induction chemotherapy, at 6 months and 12 months. Four color multiparameter flowcytometry was used for MRD quantification as previously illustrated using Navios flow cytometer (Beckman Coulter, Electronics, Hialeah, FL, USA) (Borowitz et al., 2003). All monoclonal antibodies were purchased from BD Biosciences (Franklin Lakes, NJ), Beckman-Coulter (Miami, FL), or Dako (Glostrup, Denmark). The B-ALL panels consisted of

monoclonal antibody combinations with a common backbone of CD10, CD19, and CD45 and antibodies against CD2, CD13, CD15, CD20, CD21, CD22, CD24, CD33, CD34, CD38, CD58, CD117, and/or CD123. The T-ALL panels consisted of antibody combinations with a common backbone of cytoplasmic and surface CD3 conjugated to two different fluorochromes associated with CD1a, CD2, CD5, CD7, CD10, CD34, CD56, CD99, and/or tdt. Cytoplasmic CD3 and nuclear tdt labeling were performed after IntraStain (Dako, Glostrup, Denmark) permeabilization.

#### *Statistical analysis*

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were reported as mean  $\pm$  SD, while qualitative data were reported as frequencies and percentages. Student t test was used to compare quantitative data between two independent groups. Chi square test was used to compare qualitative data between different groups. Kaplan Meier survival analysis with Log rank test were used to compare time to event between different groups. Level of confidence was set to 95% and margin of error was accepted at 5%. Differences were considered to be statistically significant when  $P < 0.05$ .

## **Results**

#### *Table 1, exhibits the clinical and laboratory profile of the patients*

The study included 70 patients. They had an average age of around  $30.8 \pm 11$  years (range 21-56). About 2/3 of them belonged to adolescents young adults (AYA) group. Most of them (62.9%) were males. Expectedly, B-ALL patients were the majority (68.6%,  $n=48$ ).

About one third of them fitted in the standard risk stratum, whereas the remainder had high risk features. Philadelphia chromosome was there in 14 patients (20%). By the end of induction, analysis of the remission status was performed in conjunction with MRD assessment for those who developed CR. Fourteen patients died during induction and 10 patients were chemo-resistant (14.3%). Forty-six patients went into CR. MRD was negative in 33/46 (72%) and positive in 13/46 (28%).

Further follow-up of these patients showed that MRD positivity was achieved in 14/32 (43.8%), and 10/28 (35.7%) patients at 6 and 12 months; respectively. By the end of one year follow up, 40% of cases enrolled were alive ( $n=28$ ) with a median survival of 85 days. Deaths were mainly due to sepsis during nadir either after induction or in the following cycles.

After dissecting patients by their MRD status at different timepoints, comparative analysis of their different demographic and clinico-pathologic features of patients and disease has been made as shown in Table 2.

In regard to post-induction MRD, it is demonstrable that the majority of patients exhibiting post-induction measurable residual disease were belonging to the older age category (age >39 years) whereas adolescent young adult group had statistically significant association to

MRD negativity with P-value 0.023. Moreover, high NCCN risk was remarkably related to positive post-induction MRD (P-value 0.034).

Despite the fact that MRD positivity was much more common among males, T-ALL immunophenotype, and

Table 1. Baseline Clinical Characteristics, Follow-up Data and Treatment Outcome of ALL Patients

Baseline clinical characteristics (N=70)	
Age (years), mean (SD), (range)	30.8 (11), (21-56)
< 39 years	66% (46/70)
>39years	34% (24 /70)
Gender	
Male	62.9% (44/70)
Female	37.1% (26/70)
Type of ALL	
B-ALL	68.6% (48/70)
T-ALL	31.4% (22/70)
WBCs (10 <sup>9</sup> /L), mean (SD), (range)	82.19 (130.25), (0.9-560)
Neutrophils (10 <sup>9</sup> /L), mean (SD), (range)	5.29 (11.32), (0.01-55)
Hemoglobin (g/dl), mean (SD), (range)	8.67 (2.72), (3.2-14)
Platelets (10 <sup>9</sup> /L), mean (SD), (range)	79.17 (56.26), (10-224)
BM blasts (%), mean (SD), (range)	84.54 (16.41), (40-98)
Peripheral blasts (%), mean (SD), (range)	51.37 (37.02), (0-99)
Extramedullary disease (+)	45.7 % (32/70)
CNS disease (+)	5.7% (4/70)
Lymph nodes or hepatosplenomegaly (+)	42.9% (30/70)
NCCN risk stratification	
Standard risk	31.4% (22/70)
High risk (Philadelphia +)	20% (14/70)
High risk (Philadelphia -)	48.6% (34/70)
Follow up after induction (N=46)	
BM blasts (%), mean (SD), (range)	11.39% (25.32), (1-90)
Minimal residual disease (MRD)	
Positive (>0.01 %)	28% (13/46)
Negative (<0.01 %)	72% (33/46)
Therapy outcome	
Complete remission	65.7% (46/70)
Refractory	14.3% (10/70)
Died	20% (14/70)
Follow up at 6 months (N=32)	
MRD	
Positive	43.8% (14/32)
Negative	56.2% (18/32)
Follow up at 12 months (N=28)	
MRD	
Positive	35.7% (10/28)
Negative	64.3% (18/28)
One-year overall survival	40% (28/70)

SD, standard deviation; ALL B, cell acute lymphoblastic leukemia; ALL, T cell acute lymphoblastic leukemia; WBC, white blood cell count; BM, bone marrow; CNS, central nervous system; NCCN, national comprehensive cancer network; MRD, minimal residual disease

Table 2. Association of Post Induction MRD with Clinical and Biologic Risk Factors (n=46)

Risk factors / Criteria	MRD+ve n, (%) (13/46), 28%	MRD -ve n, (%) (33/46) 72%	p-value
Gender			
Male	10	20	0.756
Female	3	13	
Age			
< 39 years	3	21	0.023
>39years	10	12	
Type			
B-ALL	4	25	0.234
T-ALL	9	8	
NCCN risk			
Standard risk	2	14	0.034
High risk (Philadelphia +ve)	3	9	
High risk (Philadelphia -ve)	8	10	
Extramedullary disease			
Present	10	8	0.689
Absent	3	25	

those having extramedullary disease, this did not yield any statistical value (P>0.05).

Minimal residual disease status at 6 and 12 months timepoints have been plotted against the same aforementioned variables used with post induction MRD reproducing the same results. However; MRD positivity

Table 3. Association of 6-Month MRD with Clinical and Biologic Risk Factors(n=32)

Risk factors / Criteria	MRD+ve n, (%) 14/32(43.8%)	MRD -ve n, (%) 18/32(56.2%)	P-value
Gender			
Male	10	12	0.789
Female	4	6	
Age			
< 39 years	3	10	0.034
>39years	11	8	
Type			
B-ALL	9	9	0.956
T-ALL	5	9	
NCCN risk			
Standard risk	4	10	0.045
High risk (Philadelphia +ve)	3	1	
High risk (Philadelphia -ve)	7	7	
Extramedullary disease			
Present	8	6	0.067
Absent	6	12	

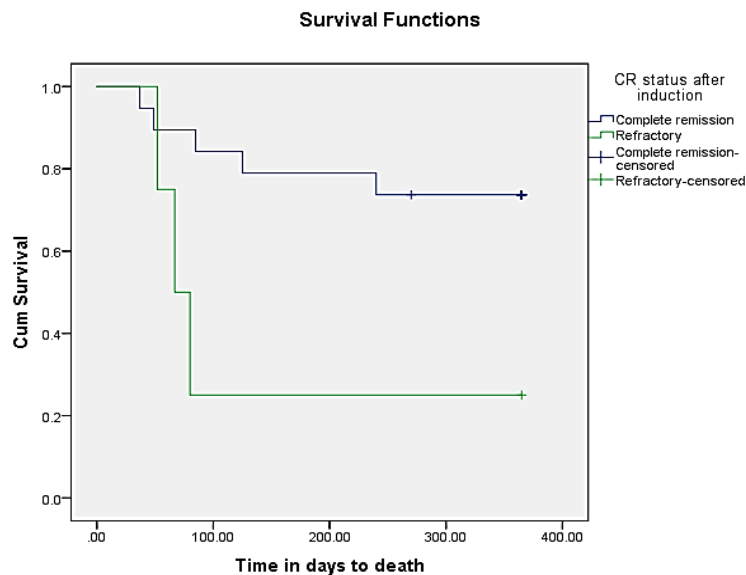


Figure 1. Overall Survival Time(OS) in Relation to Remission Status after Induction

Table 4. Association of 12-Month MRD with Clinical and Biologic Risk Factors(N=28)

Risk factors/Criteria	MRD+ve n, (%) 10/28 (35.7%)	MRD -ve n, (%) 18/28 (64.3%)	p-value
Gender			
Male	7	10	0.346
Female	3	8	
Age			
< 39 years	2	12	0.014
>39years	8	6	
Type			
B-ALL	4	13	0.002
T-ALL	6	5	
NCCN risk			
Standard risk	3	12	0.023
High risk (Philadelphia +ve)	1	0	
High risk (Philadelphia -ve)	6	6	
Extramedullary disease			
Present	3	4	0.065
Absent	7	14	

at 12 months was significantly associated with T-ALL (P 0.002). This is well-depicted in tables (3) and (4).

There is significant association between CR and negative MRD with OS (P value 0.027 in Figure 1).

### Discussion

Minimal residual disease in acute lymphoblastic leukaemia is the persistence of residual leukemic cells in the bone marrow and /or peripheral blood of the patients after receiving cytotoxic chemotherapy and radiotherapy. It is used as a way to foretell the probability of relapse and

the survival rate. Moreover, it guides the clinician decision in regard to escalating or de-escalating therapeutic intensity (Kruse et al.,2020).

Many laboratory methods are utilized to assess MRD; of them multi-parameter 3 or 4 colour flowcytometry and polymerase chain reaction (PCR) were the most extensively used techniques. Multi-parameter colour flowcytometry depends upon the detection of peculiar leukemia associated immunophenotype (Abou Dalle et al., 2020) This includes either aberrant expression of myeloid associated antigens or reduced or enhanced expression of lymphoid antigens normally expressed on benign B or T-cell progeny. However; PCR relies on the amplification of immunoglobulin or T-cell receptor gene rearrangements or recurrent genetic abnormalities (Abou Dalle et al., 2020).

In this current study, we assessed MRD in adult ALL patients after completing the induction chemotherapy, at 6 and 12 months timepoints and plotted these results against different patients as well as disease prognostic variables.

In pediatric ALL, MRD post induction range between 25-30% (Borowitz et al., 2008; Patkar et al., 2017), in our study 13 out of 46 patients (28%) had positive MRD post induction, furthermore MRD positivity is demonstrable in 14 out of 32 patients (43.8%), and 10 out of 28 patients (35.7%) at 6 and 12 months; respectively.

It is noticeable that older age category was associated with MRD positivity at different timepoints in our study. This is concordant with the results obtained by Kikuchi and coworkers who showed that age >55 years was associated with measurable residual disease on day 100 by univariate analysis. However; multivariate analysis failed to prove age as an independent predictor of positive MRD in that study (Kikuchi et al., 2010)

In our study, minimal residual disease positivity at 12 months was associated with T-ALL immunophenotype. Yet; this is not reproducible at post-induction or 6 months timepoints. No other studies have studied such an

association which may warrant further studies as it may explain the higher incidence of relapse among T-ALL cases. In addition, it may be explained by the slower pace of T-ALL disease in responding to therapy when compared to B-cell ALL (Raetz and Teachey, 2016)

In our study, minimal residual disease status was consistently associated with cytogenetic risk; with MRD positivity being linked to poor risk cytogenetics. This is concordant with the study held by Borowitz and colleagues in 2008 on childhood ALL cases. They observed that MRD positivity in patients of standard risk with poor risk cytogenetics was about double that encountered in patients with favourable cytogenetics like TEL-AML1 (Borowitz et al., 2008).

We failed to prove an association between MRD status in our cohort and extramedullary disease. This may be explained by the need of larger sample size to study such a relationship.

In our study, MRD negative cases enjoyed significantly longer overall survival in comparison to MRD positive counterparts. This mirrors the results obtained by Gökbuget et al who evaluated molecular response among Philadelphia negative ALL cases using PCR and identified molecular failure as a predictor of lower survival rates (Gökbuget et al., 2014) This is also in line with several other studies (Kikuchi et al., 2010; Berry et al., 2017; Bassan et al., 2019).

In conclusion, MRD positivity was significantly associated with older age group, high NCCN risk stratum and T-ALL immunophenotype. Patients with complete remission and negative MRD exhibited significantly higher overall survival. minimal residual disease assessment in adult ALL patients should be incorporated as an essential component of patient's follow up. This helps us to tailor therapy as per the patient's needs to achieve better disease control without undue toxicities.

## Author Contribution Statement

Rasha Ibrahim did Study Design and Statistical Analysis, Alia Saeed did Data Collection and Literature Search, all authors contributed to Data Interpretation, Manuscript Preparation.

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We acknowledge Flowcytometry lab in Clinical pathology department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

It is approved by ethical committee of faculty of medicine, Ain Shams University.

### Availability of data

The data that support the findings of this study are available on request from the corresponding author.

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