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Full Length Article

The prognostic significance of minimal residual disease in adult Egyptian patients with precursor acute lymphoblastic leukemia

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KEYWORDS

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Abstract *Background:* Minimal residual disease (MRD) studies in adult acute lymphoblastic leukemia (ALL) give highly significant prognostic information superior to other standard criteria as age, gender and total leucocytic count (TLC) in distinguishing patients at high and low risk of relapse.

Objectives: We aimed to determine the value of MRD monitoring by flowcytometry (FCM) in predicting outcome in adult Precursor ALL patients.

Patients and methods: Bone marrow (BM) samples were analyzed by 4-color FCM collected at diagnosis and after induction therapy (MRD1) to correlate MRD positivity with disease free survival (DFS) and overall survival (OS).

Results: Study included 57 adult ALL patients (44 males and 13 females) with a median age of 22 years (18–49). DFS showed no significant difference with age, gender and initial TLC (p = 0.838, 0.888 and 0.743, respectively). Cumulative DFS at 2 years was 34% for B-lineage ALL (n: 35) and 57% for T-lineage ALL (n: 18) (p = 0.057). Cumulative DFS at 2 years was

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7% for MRD1 positive (high risk, HR) versus 57% for MRD1 negative patients (Low risk, LR) ($p < 0.001$). Cumulative DFS at 2 years was 29% for HR patients (n: 26) versus 55% for LR (n: 27) according to GMALL classification ($p = 0.064$). Cumulative OS did not differ according to age, gender and TLC ($p = 0.526$, 0.594 and 0.513, respectively). Cumulative OS at 2 years was 36% for B ALL (n: 39) versus 77% for TALL (n: 18) ($p = 0.016$) and was 49% for Philadelphia chromosome (Ph) negative patients versus 0% for Ph-positive patients ($p < 0.001$). Regarding MRD1, OS at 2 years was 18% for MRD1 HR (n: 17) versus 65% for MRD1 LR (n: 38) ($p < 0.001$). OS was 35% for high-risk patients (n: 30) and 62% for low-risk patients (n: 27) classified according to GMALL risk stratification ($p = 0.017$).

Conclusion: MRD by FCM is a strong independent predictor of outcome in terms of DFS and OS and is a powerful informative parameter in guiding individual treatment in ALL patients.

Introduction

Based on retrospective analyses of large cohorts of patients, conventional pre-therapeutic risk criteria including age, elevated total leucocytic count (TLC) at diagnosis, adverse immunophenotypic features and cytogenetic as well as molecular aberrations provide the basis for upfront risk stratification in current treatment protocol [1]. The classical definition of remission in ALL based on cytomorphology provides only superficial information about the effectiveness of the treatment because, within the patient group that achieves remission, morphology is unable to discriminate between patients at high risk of relapse and those with excellent prognosis. Therefore, sensitive techniques for Minimal residual disease (MRD) detection were developed for detection of lower frequencies of malignant cells during and after treatment [2]. MRD measurement by flow cytometry (FCM) is based on the detection of leukemia associated immunophenotypes (LAP) that can be used to distinguish them from normal hematopoietic cells [1].

The source of relapse in adult precursor ALL patients is the persistence of MRD that is undetectable by standard diagnostic techniques. Several studies have shown that detection of MRD in childhood and adult ALL is an independent risk parameter of high clinical relevance, both in de novo and relapsed ALL as well as in ALL undergoing hematopoietic stem cell transplantation [3–5] and suggest that detection of MRD at an early time point during/following induction or consolidation therapy has emerged as a powerful and independent predictor of prolonged event free survival (EFS) in children and adults with ALL [6,7]. Consequently, an increasing number of treatment protocols use MRD as a tool for treatment stratification. However, the decisions for selection of one MRD methodology over another are complex and dependent upon a number of factors in our institution especially time to deliver results, expertise and resources.

In this study, we aimed to determine the value of MRD monitoring by FCM in adult precursor ALL patients especially post-induction of cytoremission in order to predict impending relapse to start preemptive salvage treatment in time.

Patients and methods

All eligible adults diagnosed as de novo precursor ALL patients who presented to the Medical Oncology Department of Egyptian National Cancer Institute (NCI), Cairo University, in the time period from April 2006 to April 2007 were recruited in this study. The study was approved by the IRB of the NCI.

Pretreatment evaluation included thorough history and full clinical examination, complete blood count (CBC), bone marrow (BM) aspiration for morphology and cytochemistry and FCM immunophenotyping. Liver and kidney functions tests, uric acid level, serum electrolytes, and cerebrospinal fluid (CSF) examination were also done in addition to cytogenetics for Ph' chromosome, chest radiographs, abdominal ultrasound, ECG and echocardiography. Informed consents were obtained from all patients before inclusion into the study.

Eligibility criteria included (1) age from 18 to 50 years, (2) all FAB subtypes except L3, (3) all immunophenotypes except Mature B subtype, (4) ECOG performance status $\leqslant 2$, (5) no other malignancy, (6) no prior chemotherapy or radiotherapy, and (7) no medical contraindications.

MRD assessment FCM was done at diagnosis to detect LAP and for detection of MRD after induction therapy (MRD1) and during maintenance therapy (MRD) using a Coulter EPICS XL-MCL flow cytometer system *(Coulter Corporation, Hialeah)* and a reagent system *(Coulter Diagnostics, Hialeah)*. Surface staining fluorescent labeled mouse monoclonal antibodies against human T, B, myeloid antigens and isotypic controls were obtained from Becton Dickenson *(Mountain View, California)*. Intracellular staining was done using IntaPrep permeabilization reagent from the Beckman Coulter by which cells were fixed with reagent 1(fixation reagent using formaldehyde), after washing, permeability was induced with reagent 2 (using Saponine for permeability) and remaining erythrocytes were lysed [8]. The following monoclonal antibodies were used for four color combinations for the detection of MRD [9]

- Precursor B-ALL: TdT/CD10/CD19/CD 45; CD10/CD20/CD19/CD 45; CD34/CD38/CD19/CD 45; CD34/CD22/CD19/CD 45; CD19/CD34/CD45; CD10/CD20/CD22/CD 45.
- T-ALL: TdT/CD1/ cyt CD3; TdT/ cyt CD3/ CD7; CD4/CD8/ CD3/CD45.

At least 3×10^5 ungated events were collected and analyzed [9]**.** Minimum target sensitivity for quantifying MRD was defined as the ability to detect 30 clustered MRD events in 3x 10^5 total cellular events (0.01%).Cut off point of MRD1 was $< 10^{-3}$ (0.1%) and for MRD at any time point was $< 10^{-4}$ (0.01%) [10,11]**.** Risk groups were defined as MRD low risk (MRD-LR) for patients with MRD $< 10^{-4}$ at all examined time

points after induction and MRD high risk (MRD-HR) for patients with MRD > 10^{-4} at any time-point.

Treatment plan

Standard (low)-risk group

(1) *Prephase* (TLC > 250x10^9/L or marked organomegaly): vincristine 2 mg I.V. (D1), prednisone 60 mg/m^2 P.O (D1–7).
(2) *Phase I induction*: vincristine 2 mg I.V. (D1, 8, 15, 22), daunorubicin (or adriamycin) 45 mg/m^2 (D1, 8, 15, 22), L-Asparaginase 5000 U/m^2 (D15–28), prednisone: 60 mg/m^2 P.O (D1–28), methotrexate 15 mg intrathecal (D1). All patients received allopurinol 600 mg/day in addition to intravenous fluids 3 L/day during induction therapy as management of hyperleukocytosis.
(3) *Cranial prophylaxis*: cranial irradiation: 24 Gy + methotrexate 15 mg intrathecal given as 4 doses (twice/week).
(4) *Phase II induction*: Cyclophosphamide: 650 mg/m^2 D 1, 14, 28 + cytosine arabinoside 75 mg/m^2 D 3, 4, 5, 6, and D 9, 10, 11, 12 and D 16, 17, 18, 19.
(5) *Phase I consolidation*: vincristine 2 mg I.V. (D1, 8, 15, 22), daunorubicin (or adriamycin) 25 mg/m^2 (D1, 8, 15, 22), prednisone: 60 mg/m^2 P.O. D1–28 in addition to triple intrathecal injection of cytosine arabinoside 40 mg, methotrexate 15 mg and dexamethasone 4 mg (D1).
(6) *Phase II consolidation: Cyclophosphamide*: 650 mg/m^2 I.V. (D 1), cytosine arabinoside 75 mg/m^2 I.V. (D 3, 4, 5, 6 and D 9, 10, 11, 12) then 100 mg/m^2 I.V. (D 25, 26, 27, 30), etoposide 100 mg/m^2 (D 25, 26, 27, 30) and triple intrathecal injection (as before) (D1).
(7) *Maintenance*: Patients classified as MRD-LR at end of consolidation therapy received maintenance treatment for two years while MRD-HR patients were planned to receive maintenance treatment for three years. Maintenance therapy included 6-Mercaptupurine 60 mg/m^2 P.O. daily, methotrexate 20 mg/m^2 I.V. once weekly and triple intrathecal therapy (as before) every 2 months.

High-risk group

(1) Prephase and induction therapy (phase I and II): as in standard risk group.
(2) Post-induction therapy HLA-typing was performed and patients were referred for allogeneic stem cell transplantation. In case of no identical donor found, patients received one course of HAM regimen (cytosine arabinoside 1.5 gm/m^2/12 h IV days 1–3, and mitoxantrone: 12 mg/m^2 IV days 3–5 with mitoxantrone given before cytosine arabinoside on day 3) then continued consolidation and maintenance treatment as in the MRD-LR group.

Statistical analysis

All analyses were performed using the statistical package for the social sciences (SPSS software 17; SPSS Inc., Chicago, USA) [12]. Analytical tests used included chi-square test for comparing two qualitative variables. Comparison of means of two groups was done by student's t-test for unpaired series and by paired *t*-test when a subject was taken as his own control. Survival analysis and analysis of duration of complete remission were done using Kaplan Meier analysis. Correlation between quantitative variables was done by the r-test diagrammatically represented by scatter dot diagram. Significance level of 0.05 was used in all statistical tests.

Results

This work included 57 adult Precursor ALL [44 males and 13 females] patients. Median age was 22 years. Thirty-seven patients had an age range from 18 to 24 years while 20 patients were between 25 and 49 years.

Patients' characteristics at diagnosis are shown in table 1

Response to induction chemotherapy

Fifty out of 57 (87.7%) patients achieved morphological complete response (CR) within 4 weeks. 4/7 patients who

Table 1 Patients' characteristics of the 57 adult precursor ALL.

Age	No (%)
Mean ± SD	24.39 ± 6.98
< 25	37 (64.9%)
25–49	20 (35.1%)
Sex	
Female	13 (22.8%)
Male	44 (77.2%)
Immunophenotyping	
B-ALL	39 (68.4%)
Pro B-ALL	2 (3.5%)
C-ALL	28 (49.1%)
Pre B-ALL	9 (15.8%)
T-ALL	18(31.6%)
Early T-ALL	5 (8.8%)
Intermediate T-ALL	11 (19.3%)
Mature T-ALL	2 (3.5%)
Leukocyte count (×10^9 L)	
< 10	14 (24.6%)
10–49	19 (33.3%)
50–100	10 (17.5%)
> 100	14 (24.6%)
BM cellularit	
Hypercellular	36(63.2%)
Normocellular	15 (26.3%)
Hypocellular	6 (10.5%)
BM morphology	
L1	5 (8.8%)
L2	34 (59.6%)
Not assessed	18 (31.6%)
Cytogenetics	
Normal	37(65%)
Philadelphia positive	7 (12.3%)
Unknown	13 (22.8%)
Initial CSF examination	
Positive for blast cells	3 (5.2%)
Negative for blast cells	54 (94.8%)

failed to achieve CR did not achieve any kind of response (i.e. *primary refractory disease*) and were all Philadelphia positive. In precursor B-ALL group, 34/39 (87.1%) achieved CR including 2/2 pro B-ALL (100%), 24/28 (85.7%) c-ALL and 8/ 9 (88.8%) pre B-ALL patients. In the T-ALL phenotype, 16/18 (88.8%) patients achieved CR within 4 weeks of induction including 4/5 (80%) early T-ALL, 10/11 (90.9%) intermediate T-ALL and 2/2 (100%) mature T-ALL phenotype. Only 3/7 Ph + patients (42.8%) could achieve CR within 4 weeks of induction therapy.

Risk stratification

According to the GMALL risk stratification [13], 27/57 patients (47%) were classified as low (standard) risk and 30/57 patients (53%) as high risk.

Evaluation of MRD

According to MRD1 positivity, 38/55 evaluable patients (69%) were classified as Low risk and 17 patients (31%) as high risk. Patients were classified according to overall MRD into high risk (MRD positive at any point) and low risk MRD (negative all through therapy). From the 55 evaluable patients, 21 (38%) were classified as *MRD-HR* and 34 (62%) were classified as *MRD-LR*.

Disease-free survival (DFS) (Table 2)

The cumulative DFS at 2 years for the whole studied group was 42% with a median of 18 months. Cumulative DFS at 2 years was 48% for L2 while 1/5 of L1 cases only remained disease free (p 0.010). Cumulative DFS at 2 years was 34% for B-lineage ALL (*n*: 35) with a median of 12 months compared to 57% for T-lineage ALL (*n*: 18) with median of 30 months ($p = 0.057$). Considering response to chemotherapy, the 2-year DFS was 44% for 50 patients who achieved CR within 4 weeks of induction chemotherapy while 2/3 patients who failed to achieve CR died. Cumulative DFS at 2 years was 57% for *MRD1-LR* (*n*: 38) (median 40 months) versus 7% for *MRD1-HR* patients (*n*: 14) (median 7 months) ($p < 0.001$) (Fig. 1). Regarding GMALL classification, Cumulative DFS at 2 years for *LR* patients (*n*: 27) was 55% (median 40 months) insignificantly higher than that of *HR* patients (*n*: 26) (29%, median 13 months) (p 0.064) (Fig. 2). Neither age, gender nor BM cellularity correlated separately with DFS ($p = 0.838$, 0.888 and 0.743 respectively).

Overall survival (OS) (Table 3)

Cumulative OS at 2 years for the whole studied group was 49% with a median of 22 months. Cumulative OS at 2 years was 20% for L1 and 55% for L2 ($p = 0.053$). Cumulative 2 years OS for B-lineage ALL was 35.6% versus 77% for T-lineage ALL ($p = 0.016$) and was 49% for Ph-negative patients (*n*: 37) versus 0% for Ph- positive patients (*n*: 7) ($p < 0.001$). Cumulative 2 year OS was 51% for patients who achieved CR within 4 weeks of induction chemotherapy compared to 29% for those who failed CR ($p = 0.0007$). The cumulative 2 years OS was 65% for *MRD1-LR* (*n*: 38) versus 18% for *MRD1-HR* (*n*: 17) ($p < 0.0001$) (Fig. ure3). Regarding GMALL classification, Cumulative OS at 2 years was higher for *LR* patients (*n*: 12); 63% versus 35% for *HR* patients (*n*: 22) (p 0.017) (Fig. 4). Neither age, gender, TLC nor BM cellularity correlated with OS ($p = 0.526$, 0.594, 0.513 and 0.551, respectively).

MRD associated risk factors

Regarding B ALL, L1 morphology showed significant association with MRD1 HR (80%) than L2 cases (26%) ($p = 0.035$). In addition, Ph chromosome positivity showed a near significant association with MRD1-HR (83%) versus Ph negative cases (35%) ($p = 0.067$). Neither age, gender, immunophenotype nor TLC correlated with MRD risk ($p = 0.644$, 1.000, 0.394 & 0.171, respectively).

Table 2 Cumulative DFS at 2 years of different ALL patients' categories.

Factor		Number of cases	Number of events	DFS		*p*-Value
				2 years (%)	Median (months)	
All cases		53	32	42.3	18	
Age	< 25	36	21	42.6	18	
	25+	17	11	41.2	16	0.838
Sex	Female	12	7	41.7	15	
	Male	41	25	42.6	19	0.888
Immunophenotyping	B-ALL	35	24	34.0	12	
	T-ALL	18	8	57.7	30	0.057
Leucocyte counts	< 50	32	19	46.5	18	
	50+	21	13	34.8	16	0.743
BM cellularity	Hyper	33	21	34.2	15	
	Normo	6	4	50.0	24	
	Hypo	14	7	57.1	30	0.633
BM morphology	L1	5	5	20.0	8	
	L2	32	16	48.0	24	0.010
Cytogenetics	Normal	37				
	Ph + ve	3				
MRD1	HR	14	14	7.1	7	
	LR	38	17	56.8	40	< 0.001
GMALL	HR	26	19	29.3	13	
	LR	27	13	55.1	40	0.064

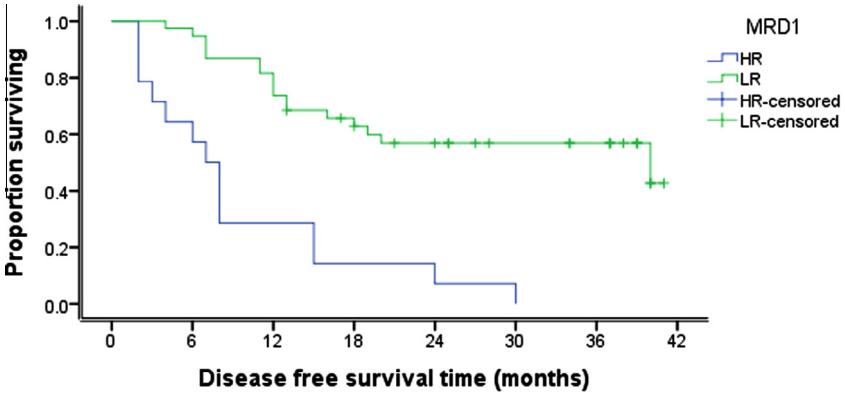

Figure 1 DFS of the 57 adult ALL patients according to MRD1.

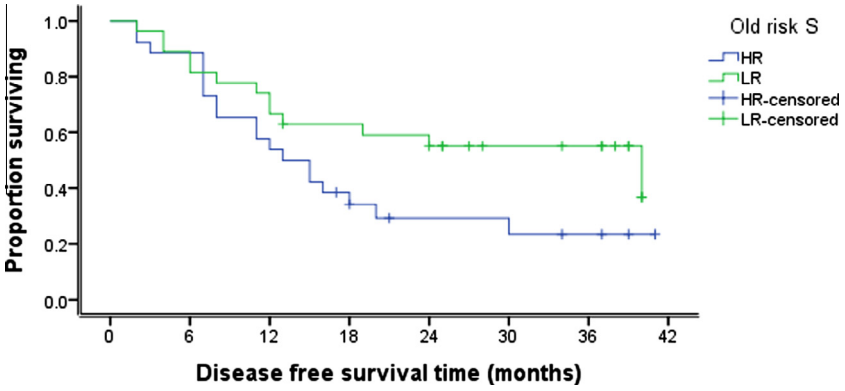

Figure 2 DFS of the 57 adult ALL patients according to GMALL classification.

Table 3 Cumulative OS at 2 years of different ALL patients' categories.

Factor		Number of cases	Number of events	OS		*p*-Value
				2 years (%)	Median (months)	
All cases		57	34	48.8	22	
Age	< 25	37	21	52.9	25	
	25 +	20	13	40.0	14	0.526
Sex	Female	13	7	53.8	25	
	Male	44	27	49.4	22	0.594
Immunophenotyping	B-ALL	39	27	35.6	15	
	T-ALL	18	7	76.9	40	0.016
Leucocyte counts	< 50	33	19	51.5	25	
	50 +	24	15	43.5	22	0.513
BM cellularity	Hyper	36	23	46.3	16	
	Normo	15	3	66.7	26	
	Hypo	6	8	60.0	40	0.551
BM morphology	L1	5	5	20.0	12	
	L2	34	18	54.7	26	0.053
Cytogenetics	Normal	37	23	48.6	22	
	Ph + ve	7	7	0 (14 m)	6	< 0.001
MRD1	HR	17	16	17.6	10	
	LR	38	16	64.8	–	< 0.001
GMALL	HR	30	22	35.0	15	
	LR	27	12	62.6	–	0.017

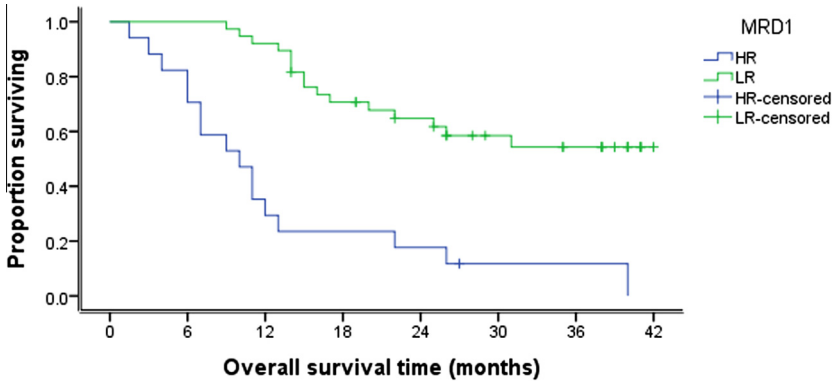

Figure 3 OS of the 57 adults ALL patients according to MRD1.

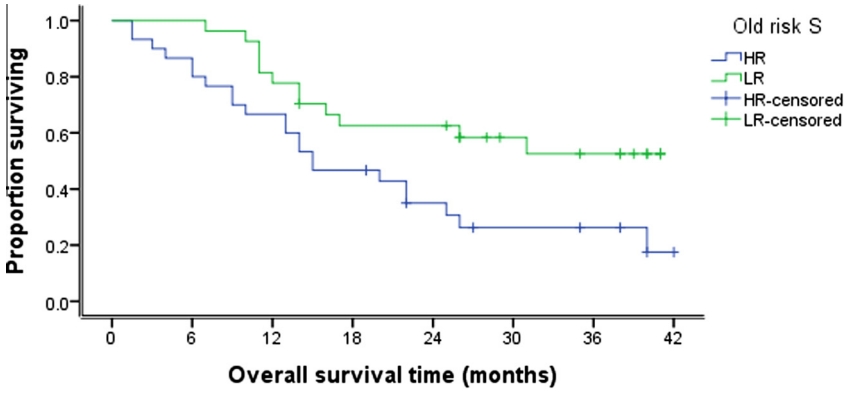

Figure 4 OS of the 57 adults ALL patients according to GMALL classification.

Mortality

Thirty-four patients died; among them 26 died from disease relapse, 2 died from fungal infections, 4 patients died from primary refractoriness, 1 patient died from fulminant HBV infection and 1 patient died from pulmonary embolism.

Discussion

Among 57 ALL patients, FCM revealed 39 patients with B-ALL (68%) and 18 (31.6%) with T phenotype which was almost comparable to others [14,15]. Precursor B-ALL patients achieved a CR rate of 87% which was similar to T-ALL patients (89%); however OS at 2 years was 36% for B-lineage versus 77% for T-lineage ALL ($p = 0.016$), a result also comparable to others [14,16]. Cumulative DFS at 2 years was 34% for B-lineage compared to 57% in T-ALL ($p = 0.057$) a result also similar to leukemia free survival (LFS) of 64% in T and 50% in B ALL Ph- negative [15]. The overall CR was 88% with a relapse rate (RR) of 60% which was comparable to CR rates reported by others [14,15]. The cumulative DFS at 2 years for the whole study group was 42% in comparison to 64% and 53% reported by others [15,17]. The cumulative OS at 2 years for the whole studied group was 49% in comparison to 43% at 3-years follow up reported by Larson et al. (1998) [18]. The cumulative 2-year OS and DFS rates for patients who achieved and failed CR were similar to others [13].

MRD is one of the most powerful and informative parameters to predict relapse and guide clinical management in ALL patients [2]. In our institution, MRD detection by FCM is relatively accurate, less expensive, applicable for most patients, rapid and having a sensitivity of at least 10^{-4}–10^{-5} with Leukemia-specificity. We found that MRD positivity decreased from 31% (17/55) after induction phase to 13% (15/46) before start of consolidation to 3% (1/33) before starting maintenance therapy. A similar decline in the percentage of MRD positivity when assessed by quantitative PCR was also reported [19]. In de novo Ph- negative ALL, post induction MRD assessment (after 2–4 months of treatment) is considered to have the most important role for evaluation of initial treatment response and MRD based risk stratification. MRD assessment after induction (after 2 weeks of treatment) additionally identifies patients with a rapid tumor clearance and a particularly good outcome. *MRD1* in our study showed that 17 patients were high risk (31%) while 38 patients were low risk (69%) with a cumulative OS at 2 years of 17% and 65%, respectively ($p < 0.001$). These results were almost similar to those reported in GMALL study with standard-risk ALL who had a rapid decline in MRD within the first month of therapy and had a 0% 3-year RR [19]. Liu et al. (2006) also found a RR of 50% for MRD positive and 7% for MRD negative patients at the end of induction [20].

Twenty-seven patients (47%) were classified as low (standard) risk (SR), while 30 patients (53%) were high risk (HR) according to GMALL risk stratification in comparison to 48%, 52%, 34%, 67% respectively reported by others [10,15]. The 2 years OS were 63% and 35% for the LR and

HR groups respectively. The 2 years DFS were 55% and 31% for LR and HR groups respectively in comparison to 87% for LR group and 51% for HR groups together in another study [15].

Concerning post remission monitoring for MRD, the GMALL proposes 3 monthly intervals for a total of 3 years as the majority of clinical relapses occur within this time [21] and reconversion to MRD positivity precedes a clinical relapse with a median time of 4.1 months between first quantifiable MRD and relapse [22]. Our results concur those of the GMALL study with high MRD ($>10^{-4}$) at any time-point associated with a RR of 66–88% [19]. In addition, cumulative DFS at 2 years was 59% for *MRD-LR* patients and 17% for *MRD-HR* with a 2 year RR of 41% and 83% respectively ($p<0.001$), a result similar to that reported by others [23]. However, in our study, DFS was significantly correlated with MRD1 but not with GMALL risk classification. Although OS was correlated with both risk classifications, the significance of FCM MRD1 positivity was higher and more informative indicating that MRD can serve as a safety net enabling early reintensification in case of MRD based treatment de-escalation. The use of MRD assessment in risk stratification of adult ALL patients may result in marked improvement in long term outcomes.

It has been previously reported that high TLC was associated with worse DFS [24], however in our study no correlation was found between survival and TLC. Seven (16%) patients were Ph-positive which was lower than the 20–30% reported [15,25]. The lower incidence of Ph-positive cases in our study may be due to lower median age of patients. CR rate in Ph-positive group (who received conventional chemotherapy not including high dose methotrexate/cytarabine or imatinib) was 43% in comparison to CR rate of 50% for Ph-positive ALL treated with imatinib and chemotherapy in another study [26]. We showed a 2-year OS of 49% achieved by Ph-negative and 0% by Ph- positive patients ($p < 0.001$) in comparison to 48% (for patients who achieved 3-log reduction in *BCR-ABL* transcripts after consolidation chemotherapy) and 0% for patients who had less than a 3-log reduction [27]. In Ph-positive ALL, the value of MRD for initial remission assessment is more limited in the era of TKI, whereas MRD assessment is frequently used for post remission monitoring. However, compared with Ph- negative ALL, relapse kinetics are more rapid with median time between MRD elevation and relapse of only 2- months with [28] and without [29] application of TKI.

In conclusion, MRD1 risk classification in adult ALL shows strong correlation with disease response and outcome in terms of DFS and OS. Our results support the usefulness of assessing MRD in patients with ALL by means of FCM; because this method is applicable to all cases and is a good option to classify and follow-up patients to decide timely therapeutic interventions. In this context, MRD can also be considered as quantitative and objective extension of established end points of hematologic remission and relapse more than a substitute of pretherapeutic risk factors.

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