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Hyper-CVAD Protocol Versus UKALL Protocol and the Minimal Residual Disease Status in Adult Acute Lymphoblastic Leukemia Patients

Ahmed Mjali[1]*, Bassam Francis Matti[2], Yassmin Ali Abdul Kareem[2], Dhulfiqar Azeez Hasan[3], Anmar Alharganee[4], Alaa Fadhil Alwan[5], Aladdin Sahham Naji[6], Tareq Abdullah Saleh[2], Amer Shareef Alhachmi[7], Ibrahim Khalil Al Shemmari[8], Haider Hasan Jaleel Al-Shammari[6], Talib Hussein Kamoona[9], Nareen Tawfeeq Abbas[10], Saja Khudhair Abbas[1] and Ahmed Ibrahim Shukr[11]

[1] Department of Hematology/Oncology, Al- Hussein Medical City, Karbala, Iraq

[2] Baghdad Teaching Hospital, Medical City, Baghdad, Iraq

[3] Teaching Laboratory, Al Sader Medical City, Najaf, Iraq

[4] Nottingham University Hospital NHS UK, Baghdad Medical City/Oncology Teaching Hospital, Iraq

[5] The National Center of Hematology, Mustansiriyah University, Baghdad, Iraq

[6] Baghdad University, College of Medicine, Baghdad, Iraq

[7] University of Thi-Qar, College of Medicine, Thi-Qar, Iraq

[8] Department of Internal Medicine and Hematology, Imamain Khadumain Medical City, Baghdad, Iraq

[9] Kufa University, College of Medicine, Najaf, Iraq

[10] Department of Hematology, Hiwa Hematology/Oncology Hospital, Sulaymaniyah, Iraq

[11] Kirkuk Oncology/Hematology Center, Kirkuk, Iraq

**Corresponding e-mail: dr_harganee@yahoo.com*

ABSTRACT

Introduction: *In Iraq acute lymphoblastic leukemia (ALL) represents the most common hematological malignancy contributing 41% of all leukemia cases. Hyper-CVAD protocol and UKALL protocol are commonly used in ALL treatment. In this study, we tried to assess minimal residual disease (MRD) post induction therapy for both hyperCVAD protocol and UKALL protocol by using 8 colors flow cytometry.* ***Material and Method:*** *Data collected retrospectively from 85 adult patients with acute lymphoblastic leukemia (≥ 14 years old) received either hyper-CVAD or UKALL chemotherapy protocol, with MRD level results post induction therapy of each protocol therapy, from January 2017 till October 2019. Fifty patients (58.82%) with B-ALL, while T-ALL were 35 patients (41.18%). Patients treated with hyper-CVAD protocol were 52 patients (61.18%) while 33 patients (38.82%) were treated with UKALL protocol.* ***Results:*** *Patients with de novo ALL of Philadelphia chromosome negative, male to female ratio 2:1 and the mean age was 23 years. Thirty seven patients (43.53%) achieved MRD negative. Of these MRD negative patients, 14 patients (37.84%) were post UKALL while 23 patients (62.16%) were post hyper-CVAD protocol with (p=0.9).* ***Conclusion:*** *Both hyper-CVAD and UKALL protocol therapy in adult ALL have a good efficacy without significant difference in achieving MRD negativity.*

Keywords: Minimal residual disease, Hyper-CVAD protocol, UKALL protocol

INTRODUCTION

Acute lymphoblastic leukemia (ALL), is a clonal expansion of hematopoietic blasts, it is an extremely heterogeneous disease that including different entities for which special therapeutic approaches are needed. In developed countries ALL represents approximately 12% of all childhood and adult leukemia and about 60% of diagnosed patients were under 20 years of age. In Iraq, leukemia represents more than 6% of cancer patients and ALL represents the most common hematological malignancy contributing 41% of all leukemia cases [1-3].

The outcomes of ALL treatment have been a successful story in pediatric oncology, while in adults were inferior. Poor results in adults ALL has been variously due to high prevalence of high-risk leukemia with increase drug resistance, reduced tolerance and treatment compliance, reluctance to accept certain temporary toxic effects and less effective treatment regimens as compared with childhood ALL [4]. The successful response in pediatric protocols leads to inspire these protocols in treatment of adult ALL patients. UKALL 2003 is the most common pediatric protocol used in Iraq which proved to be effective up to 24 years old [5-7]. These protocols use non-myelosuppressive drugs, such as glucocorticoids and L-asparaginase and fewer myelosuppressive drugs, such as anthracycline, but L-asparagines side effects was a great concern [6].

Current intensive treatment protocols such as hyper-CVAD is widely used in many centers and have improved the outcomes in adults ALL patients, with more than 80% response rate, nonetheless we still have to substitute our strategies to increase the rates of long-term survival which range from 30 to 45% [8,9].

Minimal residual disease (MRD) represents the low level disease that traditional morphology cannot detect. MRD monitoring can be a way to accurately predict early response to treatment and detect persistence disease in ALL patients [10,11]. Nowadays, the most important technique to assess MRD in ALL are Flow cytometric analysis (FCM) and Polymerase chain reaction (PCR) [12].

The assessment of MRD by flow cytometry needs to identify immune phenotype markers with selected positive expression in leukemia cells comparable to negative expression in normal hematopoietic lineage cells [13,14]. Differences in gating strategies, antibody panels and the applied immune-staining protocols are significantly different according to centers and chemotherapy protocols making MRD as subjective expert procedures [15]. Here in this study, we tried to assess MRD post induction therapy of both hyper-CVAD protocol and UKALL protocol by using 8 colors flow cytometry, supposing the differences in efficacy degree on MRD status of each protocol.

PATIENTS AND METHODS

Patients Selection

This is a retrospective analysis from different Iraqi centers experience with MRD status data in adult ALL patients between January 2017 and October 2019. We included all data for ALL patients ≥ 14 years from both genders. All patients were Philadelphia chromosome negative without significant co-morbidity and good performance status by ECOG scale (less than 3). On other side we excluded patients who previously treated or relapsed, patients with Burkitt lymphoma/leukemia, patients with significant co-morbidities or poor performance status by ECOG scale (≥ 3), patients with Philadelphia chromosome positive and those with CNS involvement at diagnosis to avoid any additional risk.

Data were obtained from the records of each patient. ALL patients in our study were treated either with hyper-CVAD protocol or UKALL protocol, according to hematologists experiences and preferences. Protocols details shown in Tables 1 and 2.

Table 1 Details of hyper-CVAD protocol [9]

Phase and Therapy	Dose	Route of Administration	Days Administered
Course 1, 3, 5, 7			
Cyclophosphamide	300 mg/m^2	b.i.d IV (2 hours infusion)	D1-3
Vincristine	2 mg	IV	D4+11

Doxorubicin	50 mg/m^2	IV (2 hours infusion)	D 4
Dexamethasone	40 mg	I.V. or oral	D1-4 , 11-14
Course 2, 4, 6, 8			
Methotrexate	1000 mg/m^2	Iv (24 hours infusion) With folinic acid rescue	D1
Cytarabine	2000 mg/m^2	b.i.d IV (2 hours infusion)	
CNS prophylaxis			
Methotrexate	12 mg	IT	D2 of each course
Cytarabine	100 mg	IT	D7 of each course
*All courses with G-CSF support, repeated every 3 weeks			

Table 2 UKALL protocol details [16]

Phase and Therapy	Dosage	Route of Administration	Days Administered
Phase 1, weeks 1-4			
Daunorubicin	60 mg/m^2	IV	D1, 8, 15, 22
Vincristine	1.4 mg/m^2	IV	D1, 8, 15, 22
l-asparaginase	10000 U	IV or IM	D17-28
Prednisone	60 mg/m^2	PO	D1-28
Methotrexate	12.5 mg	IT	D15
Phase 2, weeks 5-8			
Cyclophosphamide	650 mg/m^2	IV	D1, 15, 29
Cytarabine	75 mg/m^2	IV	D1-4, 8-11, 15-18, 22-25
6-Mercaptopurine	6 mg/m^2	PO	D1-28
Methotrexate	12.5 mg	IT	D1, 8, 15, 22
Intensification/CNS prophylaxis (3 cycles)			
Methotrexate	3000mg/m^2	IV (with folinic acid rescue)	D1,8,22
Asparagines	10000 IU	IV	D2, 9,23
*Followed by transplantation or consolidation and maintenance therapy			

Response Assessments and MRD

MRD was tested by flow cytometry analysis for the bone marrow aspiration; as the MRD levels in B-cell precursor ALL tend to be 1 to 3 logs lower in peripheral blood than in bone marrow [11]. European clinical guidelines and protocols recommend testing patients who achieve a complete hematological remission (CR) for MRD for the purpose of risk stratification which drives treatment decisions and level ≥ 0.01% or 10^{-4} cell considered as MRD positive [17,18]. In general, the assessment made at the end of induction therapy when the peripheral blood indices recovery (hemoglobin ≥ 10 g/dl, ANC ≥ 1500 mml and platelets count ≥ 100 × 10^9 without blasts in peripheral blood) [19,20].

It is important to note that the first sample taken from the bone marrow should be send for MRD analysis, to prevent dilution with peripheral blood (as dilution may lead to underestimation of MRD). The bone marrow sample/peripheral blood collected in either EDTA or heparin. The sample transported at room temperature and the assay was complete within 48 hours. Before starting the staining, it is important to get the cell counts on the sample to be processed. The bone marrow sample can be directly run on an automated cell counter to get the counts. It is optimal to start with 1^{-2} × 10^6 cells in 100 µL sample volume [21].

The samples are all processed by a Stain-lyse-wash approach and the same is followed for MRD analysis by 8 color flow cytometry from BD. Red cells lysis is performed using a fixative containing lysing reagent like buffered NH_4Cl containing 0.25% formaldehyde. Since cytoplasmic/nuclear markers are also used, the tube after surface staining is

premeabilised using commercial permeabilising reagent as per company specific protocol, cytoplasmic antibodies are added. After the optimal staining time, the cells are washed once, the volume is brought to 500 µL sheath and cells are ready for acquisition [21]. Regarding gating markers, in our lab we use the protocols that shown in Tables 3 and 4.

Table 3 Eight colors panel used in the detection of MRD for B-ALL [12,22]

	Florochromes	V450	v 500	FITC	PE	PerCP Cy 5.5	Pe-Cy7	APC	APC H7
TUBE 1	CD Marker	CD20	CD45	CD58	CD66c	CD 34	CD19	CD10	CD38
	Clone	L27	2D1	1C3	B6.2	8G12	SJ25C1	HI0A	HB7
TUBE 2	CD Marker	CD73	CD45	CD123	CD200	CD34	CD19	CD10	CD81
	Clone	AD2	2D1	7G3	MRCOX104	8G12	SJ25C1	HI0A	JS81

Statistical Analysis

The results were analyzed statistically in all tables, and Chi square procedure was used to find the significant differences at the level of significance ($p \leq 0.05$).

Table 4 Eight colors panel used it in the detection of MRD for T-ALL [12,22-24]

Tube 1	CD3	CD7	CD19	CD5	CD34	cytCD3	CD38	CD45
Tube 2	CD3	CD7	TdT	CD8	CD16/56	cytCD3	CD4	CD45
Tube 3	CD3	CD7	CD117	CD99		cytCD3	CD1a	CD45

RESULTS

Patients Characteristics and Treatment Protocols

In this study, there were 85 patients with de novo ALL and Philadelphia chromosome negative, of these patients, 57 patients (67.06%) were male and 28 patients (32.94%) were female, with a male to female ratio 2:1. Mean age was 23 years, most of the patients (48%) with age group between 12-19 years old. There were 50 patients (58.82%) with B-ALL, while T-ALL were 35 patients (41.18%). Fifty two patients (61.18%) were treated with hyper-CVAD protocol while 33 patients (38.82%) were treated with UKALL protocol. Most of patients ≥ 25 years treated with hyper-CVAD protocol (22 patients) while only 5 patient ≥ 25 years treated with UKALL protocol.

Minimal Residual Disease Status

From all cases, there were 37 patients achieved MRD negative, 14 patients (37.84%) achieved MRD negativity post induction with UKALL protocol while 23 patients (62.16%) post induction with hyper-CVAD protocol with (p=0.9) as shown in Table 5.

Table 5 Relationship between type of protocol and MRD status

MRD	Protocol		Total	p-value
	UKALL	Hyper-CVAD		
Negative	14 (37.84%)	23 (62.16%)	37 (100%)	0.9
Positive	19 (39.58%)	29 (60.42%)	48 (100%)	0.9

MRD in Related to Age, Gender, Type of ALL after Induction

By analysis of MRD status according to the age, gender and different types of ALL patients, there was no significant difference between MRD status regarding age and gender. Regardless protocol therapy, T cell type ALL tend to

be more MRD positive with 29 patients (82.86%), while only 19 patients (38%) of B-ALL stay with MRD positive (p=0.0001) as shown in Table 6.

Table 6 Assessment of age, gender, type of ALL regarding to MRD

Variable		MRD Negative	MRD Positive	RR (95%CI)	p-value
Age	<25	23 (39.65%)	35(60.35%)	1.52	0.9
	≥ 25	14 (51.85%)	13(48.15%)	0.92	0.9
Gender	Female	13 (46.43%)	15 (53.57%)	1.15	0.9
	Male	24 (42.11%)	33 (57.89%)	1.37	0.9
Type of All	B Type	31 (62%)	19 (38%)	0.61	0.1
	T Type	6 (17.14%)	29 (82.86%)	4.83	0.000*
Type of Protocl	Hyper-CVAD	23 (44.23%)	29 (55.77%)	1.26	0.9
	UKALL	14 (42.42%)	19 (57.58%)	1.35	0.9
*means the significant differences, Relative Risk (RR), Confidence Interval (CI)					

Types of ALL Regarding Protocol Types and MRD

Nineteen patients (61.29%) with B-ALL post induction therapy with Hyper-CVAD protocol were MRD negative while 12 patients (38.71%) were MRD negative post induction therapy with UKALL protocol (p=0.900). In T-ALL patients 4 patients (66.7%) were MRD negative post induction treatment with Hyper-CVAD while only 2 patients (33.3%) were MRD negative post induction treatment with UKALL protocol and (p=0.900) as shown in Table 7.

Table 7 Assessment of type of ALL regarding to protocols and MRD status

	Protocol Type	Negative MRD	Positive MRD
B-ALL	Hyper-CVAD	19 (61.29%)	11 (57.89%)
	UKALL	12 (38.71%)	8 (42.11%)
	Total	31 (100%)	19 (100%)
p-value		0.9	0.9
T-ALL	Hyper-CVAD	4 (66.67%)	18 (62.1%)
	UKALL	2 (33.33%)	11 (37.9%)
	Total	6 (100%)	29 (100%)
p-value		0.9	0.9

DISCUSSION

Although clinical factors and cytogenetics play an important role in guiding therapy, MRD has become standard practice to evaluate patients for using molecular techniques such as flow cytometry and PCR [25]. The first studies on MRD detection in ALL date back from the 1980s, using immunofluorescence microscopy. Three decades later, monitoring of MRD has become routine clinical practice in frontline treatment of ALL patients and guide treatment decision [15,20,26].

In our study from 85 adult ALL patients, there were 37 patients (43.53%) achieved MRD negative post induction therapy. For those who achieved MRD negative, 37.84% of them were post induction with UKALL protocol and 62.16% were post hyper-CVAD protocol with no significant statistical differences. Siegel et al, accounted six comparisons of disease control with hyper-CVAD and pediatric regimens, two were reports of comparable outcome and four reported inferior outcome with hyper-CVAD [27]. From 52 patients who received hyper-CVAD protocol 23 patients (44.23%) were MRD negative. This was close to study done by Cassaday, et al. where 42% of patients were MRD negative at 1st assessment [19].

In our study there were 42.4% patients achieved MRD negativity post UKALL protocol without any significant dif-

ference between MRD status and age. While in other studies for patients who received UKALL protocol showed 52% had MRD negative and those with aged ≥ 16 years old were more MRD high risk compared to younger age groups [7,28].

The T-ALL patients were tend to be more MRD positive with 29 patients (82%), while only 19 patients of B- ALL (38%) stay MRD positive (p=0.000). Our result was consistent with previous studies that suggest those patients with T-ALL had a poorer event free survival and an inferior overall survival than patients with other leukemia subtypes. It is generally associated with high levels of MRD during and at the end of remission induction therapy [20]. In the same time most of the patients (48%) with age group between 12-19 years old and older adolescents were more likely to have T-cell ALL and detectable minimal residual disease during or at the end of remission induction compared with younger patients [29].

CONCLUSION

Hyper-CVAD and UKALL protocols in adults ALL are effective as same as regarding achieving MRD negativity without significant difference. Subsequent MRD follow up after the end of treatment is advisable to assess the stability of response status of each protocol therapy.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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