Clinical Medicine and Health Research Journal (CMHRJ)
Volume 03, Issue 02, March - April, 2023
Page No. 346-354

Research Article

Study the Relationship Physiology and Molecular Biology in Patients of Iraq Drug Use Parameters Acute Leukaemia (AL)

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Received: 01 March, 2023 Accepted: 03 April, 2023 Published: 08 April 2023

Abstract:

Study of paper is physiology use to analysis total in LDH is an intracellular enzyme whose activity is associated with cellular metabolism, WBCs count in blood to patients which makes it a marker of cell proliferation and cell injury and study with molecular biology of marker parameters use drug the presence of survivin in neoplastic cells contributes to tumor development and its high transcription has been reported as a predictor of poor prognosis in several hematological malignancies. However, despite being frequently present in ALs, the impact of survivin transcription in this type of hematologic malignancy remains undefined. Some studies report that survivin predicts an unfavorable clinical outcome in ALs, while others do not. In the present study, despite the small number of patients evaluated, the transcription of survivin at diagnosis did not seem to be related to a worse response to treatment or a worse prognosis. This is because, of the six patients who expressed survivin, three are in complete remission and, of the three who died, two were already considered to have an unfavorable prognosis regardless of survivin transcription, one due to advanced age and the presence of leukocytosis in the liver. Leukometry at diagnosis was another prognostic factor evaluated in this study. Patients diagnosed with AL may have elevated, normal, or decreased WBC counts at the time of diagnosis and, in general, counts greater than 30,000 WBCs/mm3 are considered to have a poor prognosis.

Keywords: physiology (LDH,WBCs) , molecular biology , drug use parameters , acute leukaemia (AL).

Introduction

Leukemias are cancers of the white blood cells, or hematopoietic stem cells, found in the bone marrow and characterised by unchecked cell growth, survival, and aberrant differentiation (BM). There were an estimated 350 thousand new cases of leukaemia worldwide in 2008, and 257 thousand deaths from the disease (Terwilliger, 2017). The financial costs for the diagnosis and treatment of neoplasms are increasingly high, and, considering that in Iraq, about 80% of the health system, including cancer care, is financed by public resources, cancer is consolidated as an important public health problem. Thus, increasing the effectiveness of treatments with the use of individualized and specific therapeutic protocols can result in a decrease in hospital stay, a decrease in costs for the Unified Health System (SUS) and an improvement in the quality of life of these patients (Dalton, 2017).

The classification of acute leukemias, based on the morphological examination of blood and bone marrow cells with the help of histochemical techniques, allows the recognition of two large groups: acute lymphoblastic leukemias (ALL) and myeloblastic leukemias. or acute non-lymphoblastic leukemias (ANL). Perhaps the term myeloblastic is restrictive to describe a group of leukemias that includes predominantly monoblastic, erythroid, or megakaryocytic forms, which is why the term LNLA is increasingly used. The incorporation of immunophenotyping methods, chromosomal analysis and molecular biology techniques allow a more precise characterization of the leukemic cell and, therefore, a more accurate subclassification of acute leukemias. Of the many proposed classifications, the FAB classification is currently the most widely accepted, particularly in ALN, while the immunological classification is more commonly used in ALL. Although the FAB classification, proposed in 1976 by a group of expert French, American and British cytologists, has been revised and updated on several occasions, some leukemias are still unclassifiable (Brunning, 2003).

Materials and Methods

The study, consecutive samples of peripheral blood or bone marrow with EDTA were collected from patients of both genders, with suspected AL and who had been treated at Baghdad Teaching Hospital (BTH), Iraq, in the period from September 2022 to March 2023. Before collecting the samples, the Free and Informed Consent Term (ICF) was signed, according to the Ethics Committee for Research in Human Beings at BTH.

In total, consecutive samples were collected from 50 patients with suspected AL. However, only patients with a final diagnosis of AL were included in the study, without a history of previous treatment of AL and who had their medical records available for consultation. Thus, nine patients who were not diagnosed with AL, three patients with recurrence of AL and five patients who did not have their medical records searched were excluded from the study, as they were not available. Therefore, 50 samples of patients diagnosed with AL were included in the study, of which 25 were from peripheral blood and 25 from bone marrow. The diagnosis of

AL was based on the morphological, immunophenotypic, cytogenetic and molecular evaluation of the leukemic cells. Subclassification was performed according to the World Health Organization Classification criteria for Hematopoietic and Lymphoid Tissue Tumors.

To verify the role of the *mdr*/MDR profile as a tool for detecting minimal residual disease, OM samples were collected from the patients included in the present study after the end of the first cycle of induction chemotherapy. Altogether, it was possible to evaluate the expression levels of MDR genes and proteins in 25 samples of bone marrow.

In addition to molecular biology and flow cytometry assays, a search was performed on medical records to obtain clinical data and other laboratory data from patients. The following data were collected from the medical records: age, WBC and LDH levels at diagnosis, gender, immunophenotype, karyotype, final diagnosis, occurrence of deaths and presence or absence of MRD after induction therapy. The absence of MRD was considered to be the occurrence of complete hematological remission, that is, the presence of 5% or less of blasts in the OM, reestablishment of normal hematopoiesis (return to normal values of hemoglobin, leukometry, differential leukocyte count and platelet count). and absence of extramedullary disease after induction therapy.

Controls of Molecular Biology Assays

Human AL cell lines were cultured and used as positive controls for RT-PCR assays. As a positive control for the expression of *abcb1*/ABCB1, the AML strain of human origin K562-Lucena was used. The Jurkat human ALL lineage was used as a positive control for the expression of abcc1/ABCC1 and *lrp*/LRP. As a control of survivin transcription and the presence of TL-2 the AML strain of human origin K562 was used. For translocations TL-1, TL-3 (TL-3) and TL-4 (TL-4), the AML strain of human origin was used as a positive control. Kasumi1, NB4 and ME-1, respectively.

Cells were maintained in appropriate cell culture bottles containing DMEM (Dulbecco's Modified Eagle's Medium) or RPMI1640 (Roswell Park Memorial Institute 1640 Culture Medium) medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate and 10 mM HEPES buffer (4-(2-hydroxyethyl)1-piperazinyl-ethanesulfonic acid) at pH 7.4. The cells were kept in an oven with a humid atmosphere at 37 ºC containing 5% CO_2. To carry out the experiments, the number of viable cells was evaluated by the Trypan Blue dye exclusion method (0.5%) and only samples with viability greater than 80% were used.

Complementary DNA Synthesis

RNA quantification was performed by fluorimetry using the Qubit™ Quantitation Platform (Invitrogen) and its quality was assessed by visualizing ribosomal RNA (rRNA) bands in 2% agarose gel electrophoresis. Good quality samples were considered to be those that did not show a trace below the rRNA bands and whose 28S rRNA band had twice the intensity of the 18S rRNA band.

For cDNA synthesis, from 1 µg to 5 µg of total RNA were added in 0.2 mL microtubes. Possible contaminating DNA in the samples was eliminated by DNase digestion. To this end, 1 µL of DNase (1 U/µL), 1 µL of reaction buffer and water treated with diethyl pyrocarbonate (DEPC-water) were added to the total RNA until the volume was 10 µL. After 15 minutes digestion at room temperature, DNase was inactivated by 1 µL of EDTA (25 mM) and incubated for 10 minutes at 65 °C.

After DNase inactivation, the samples were subjected to a 5-minute incubation at 70 ºC, followed by an incubation on ice for 5 minutes. After incubation on ice, 15 µL of mix solution was added to each sample tube containing: 5 µL of 5x first strand buffer (250 mM Tris-HCl, pH 8.3; 375 mM KCl; 15 mM MgCl2), 2 µL of DTT (0.1M), 2 µL of random hexamers (100 ng/µL), 0.4 µL of dNTP mix (100 mM each), 0.5 µL of RNAse (40 U/µL), 0.5 µL of reverse transcriptase (200 U/µL) and water-DPEC q.s.p. to 15 µL. The samples were then taken to the thermocycler (Mastercycler Personal, Eppendorf) and subjected to the following schedule: 25 °C for 5 minutes, 37 °C for 60 minutes and 90 °C for 5 minutes. At the end of programming, the samples were incubated on ice for 5 minutes. The cDNA samples were measured by fluorimetry using the Qubit™ Quantitation Platform (Invitrogen) and stored in a freezer at minus 20 ºC to be later used in assays to detect the transcription of resistance genes and to investigate the presence of chromosomal translocations.

Detection of Gene Transcription of *abcb1*, abcc1, *lrp* AND survivin BY Semi quantitative RT-PCR

The evaluation of gene transcription of resistance proteins was performed using the semiquantitative RT-PCR technique. The oligonucleotide primers or primers pairs (Table 1) used to assess the transcription of *abcb1, abcc1, lrp and survivin* were previously described by Tomiyasu, et al. (2012) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcription was used for band normalization.

Table 1 : Sequence of primers for the detection of *abcb1, abcc1, lrp* and surviving

Gene	Sequencia	product size
abcb1	5'–CCGCAAGTTTACCTGCAGG–3'	157bp
	5'–GCTCCTAACTTCTGAGGTTCA–3'	
abcc1	5'–TGGGGGACTGTCACGAAT–3'	260bp
	5'–AGGCCGAATATGACTTCCC–3'	
lrp	5'–GTCGGGCAGCTCGTCGGTGTTCTG–3'	240bp
	5'–GCCTGGGTCTGTCTCTTGCCTTGG–3'	
survivin	5'–GCATCCTGGACTTCTCAACACC–3'	393bp
	5'–CCCATGGAGCTGCATCAGCCA–3'	
GAPDH	5'–CCCGTCTTCGAGAAACCATGA–3'	330bp
	5'–TCTGGGATGTATGAGGCAGG–3'	

All reactions were prepared with a final volume of 50 µL. PCR reactions with primers for *abcb1* and *lrp* were performed. The samples were initially denatured at 94 °C for 5 minutes and then subjected to the ideal reaction conditions for each pair of primers. At the end of the PCR cycles, a final extension was performed at 72°C for 10 minutes. The ideal reaction conditions and the number of cycles used were established in previous tests carried out at the Laboratory of Experimental

Oncology and Hemopathies (LOEH) so that the PCR ended in the exponential phase of the amplification.

Research of Translocations TL-1, TL-2, TL-3 & TL-4

The search for chromosomal translocations was performed using the RT-PCR technique followed by nested PCR. The primers (Table 2) and the PCR conditions used for the investigation of chromosomal translocations were those described by the BIOMED-1 program.

For the first PCRs of all chromosomal translocations, primers coded as A (sense) and B (antisense) were used. To avoid releasing false positive results, in parallel with the first PCR, a confirmatory PCR was performed with primers C (sense) and E3' (antisense), with the exception of TL-1 and inv (16)(p13;q22) in which the confirmatory primers used were those coded as E5' (sense) and D (antisense). Nested PCRs were performed using primers encoded with C (sense) and D (antisense).

Table 2: Sequence of primers for the investigation of chromosomal translocations

translocation	Primer	Sequence
TL-1	AML1-A	5'–CTAACACCCATGACGCAGAGC–3'
	ETO-B	5'–AGAAGACCCATTGGGAAGGCT–3'
	AML1-C	5'–ACCTCAGATGGGGTCGTTTGTC–3'
	ETO-D	5'–GCTTGAACTCCTCTTGGTTGGA–3'
	AML1-E5'	5'–TGGCGGGACTACCTAATTGAATAA–3'
TL-2 p190	BCR-e1-A	5'–GGCTCACAGAACTGCACAATG–3'
	ABL-a3-B	5'–ATTCCTCGTTTGGACACGTCC–3'
	BCR-e1-C	5'–CAACAGCGCCTAGAACTTCTC–3'
	ABL-a3-D	5'–TTTTGTGATCCCCAGCCTATATA–3'
	ABL-a3-E3'	5'–TGTTGCTTGCGTGAGACTGTGTA–3'
TL-2 p210	BCR-b1-A	5'–GATCAGAATCAGTGTTTCCGCT–3'
	ABL-a3-B	5'–CTTCATTCCACGTTTGGGACC–3'
	BCR-b2-C	5'–CTCGTTGGTCAGATGCACCAA–3'
	ABL-a3-D	5'–TATATCGTGGCCCCATTCTATAT–3'
	ABL-a3-E3'	5'–TGACTAGTTGGCGTGTGCTTATG–3'
TL-3	PML-A1	5'–CCTTCTTACCCATCAAGTGGC–3'
	PML-A2	5'–CTGGTGGACTGAGGCTGC–3'
	RARA-B	5'–GCGTAGCTTGGGTAGGATGA–3'
	PML-C1	5'–TCAGGAAGATGAGGAGTCTGG–3'
	PML-C2	5'– AGGCTACGAGGAGATCGCA–3'
	RARA-D	5'–CGTCTCTCAATTGCTGCTGG–3'
	RARA-E3'	5'–GCCCACTTCAAAGCACTTCT–3'
TL-4	CBFB-A	5'–GCAGAAGGTATGCAAGGTATTG–3'
	MYH11-B1	5'–TGAACTGTTGGGCTCCGCAA–3'
	MYH11-B2	5'–TCATCCTGCTCTCTTCTCCTCT–3'
	CBFB-C	5'–GGATGGGGAGTTCTTTCTGG–3'
	MYH11-D1	5'–TCTGTCTTGACGCTCCCAAC–3'
	MYH11-D2	5'–CTTGAGAGCTGTTCCTGC–3'
	CBFB-E5'	5'–CAGACAAACAGGGACAGACGA–3'

DNA Extraction

DNA extraction was performed using a 5 M guanidine isothiocyanate solution. For that, 200 µL of peripheral blood or bone marrow were transferred to a microtube containing 1 mL of 5 M guanidine isothiocyanate solution and subjected to vortex homogenization. The sample was then kept under continuous stirring at room temperature for 12 hours overnight. After incubation, 50 µL of acidified silica dioxide solution was added to the microtube and the sample was homogenized by inversion for 5 minutes. Afterwards, the sample was centrifuged at 1000 g for 1 minute at room temperature and the supernatant was discarded. The DNA-containing silica pellet was then washed twice with 500 µL of 5M guanidine isothiocyanate wash solution, twice with 500 µL of 70% ethanol and once with 500 µL of ultra pure acetone. Between each wash, the supernatant was removed after the sample was centrifuged at 1000 g for 1 minute at room temperature. At the end of the last wash, acetone residues were removed by evaporation in a thermoblock (Thermomixer compact, Eppendorf) at 56 °C for 10 minutes. The sample was rehydrated with 25 µL of Tris-EDTA Buffer (10 mM Tris.HCl, pH 6.4; 1 mM EDTA, pH 8.0) and again incubated in thermoblock at 56 °C for 10 minutes under agitation. Afterwards, the sample was centrifuged at 2,600 g for 5 minutes and the supernatant containing the genomic DNA was transferred to a new microtube with a capacity of 0.6 mL and frozen at minus 20 °C for later use in the assays for mutations in genes FMS3 and JAK2.

Statistical Analysis

The creation of the database and the statistical analysis were performed using SPSS version 17.0® and MedCalc® version 12.3.0.0 software. Demographic data were summarized as

absolute numbers and percentages in the case of nominal variables; and median of maximum and minimum values in the case of numeric variables. The expression of multidrug resistance proteins (ABCB1, ABCC1 and LRP) were numerically summarized through median and maximum and minimum values (thresholds). The presence of FMS3-DIT, FMS3-D835 and JAK2V617F mutations, as well as survivin translocations and transcription were summarized dichotomously (present or absent).

To evaluate the distribution of the samples, the Shapiro-Wilk or Kolmogorov-Smirnov tests were used. Independent numerical variables were compared between groups using the Mann-Whitney U test or the Kruskal-Walli's test. The comparison of two dependent numerical variables between the groups was performed using the Wilcoxon test. The correlation between two numerical variables was performed using Pearson's correlation and the association between two or more nominal variables was verified using the chi-square or Fisher's exact test. The survival curve was constructed using the Kaplan-Meier method. A significance level of 5% ($P<0.05$) was considered.

Results and Discussion

In this study, 50 patients diagnosed with AL were evaluated. Of these, 25 (53.3%) were male and 25 (46.7%) were female and the median age was 41 years (15-87 years; Table 1). Regarding the AL subtype, 27 (62.6%) cases of AML, 23 (34.6%) of ALL and two (2.8%) of AL were diagnosed. The distribution of AL subtypes according to WHO criteria was as follows: AML (one case; 1.3%), AML with minimal differentiation (one case; 1.3%), AML without maturation (five cases; 6.7 %), maturing AML (six cases; 8.0%), maturing AML with TL-1 (three cases; 4.0%), acute myelomonocytic leukemia (three cases; 4, 0%), acute monoblastic leukemia (two cases; 2.7%), acute monocytic leukemia (two cases; 2.7%), acute erythroblastic leukemia (one case; 1.3%), acute megakaryoblastic leukemia (one case; 1.3%), acute promyelocytic leukemia with t(15;17)(q22;q12). As can be seen in Table 3, which presents the clinical and laboratory characteristics at the time of diagnosis of the patients included in the study, the patients who did not go into remission were those who had a significantly higher median age (54 years) than those who went into remission. remission (35 years) ($P<0.001$). The medians of leukometry, percentage of blasts and LDH at diagnosis were similar between the two groups of patients. In addition, there was also no association between gender and response to treatment.

Of the total number of patients analysed (n=50), only three had the FMS3-DIT mutation (Table 3). One was diagnosed as having APL, another as a non-mature AML, and the third as a common-type ALL-B. Patients with APL and AML without maturation achieved remission after treatment. However, the patient diagnosed with common type B-ALL died without going into remission. The four patients who were positive for the FMS3-D835 mutation went into remission after treatment and were alive until the end of the present work. Two of them were diagnosed as AML with maturation with TL-1, one as APL and another as myelomonocytic AML. No association was observed between treatment response and the presence of FMS3 mutations. In addition, no patient had the JAK2V617F mutation. Regarding the research of translocations, three patients presented TL-1, 19 to TL-5 and five to t(9;22)(q34;q11.2) (denoted as TL-2.2), receiving the diagnosis of AML with maturation with TL-1, APL with TL-5 and ALL-B with TL-2.2, respectively. No patient had TL-4. In addition to these genetic alterations, the transcription of survivin, an anti-apoptotic protein, in the leukemic cells of these patients was also investigated. Due to the insufficient amount of sample or the absence of amplification of the constitutive gene (gapdh), it was not possible to assess the transcriptional intensity of survivin in 11 cases. As can be seen in Table 3, six patients expressed survivin at the time of diagnosis. Of these, one had mature AML, one had acute monoblastic leukemia, two had common type-ALL-B and two had T-ALL. Patients diagnosed with maturation AML, acute monoblastic leukemia and T-ALL died. There was no association between the presence of MRD and survivin transcription.

The median survival of patients with AML was three months, those diagnosed with B-ALL at 25 months and those diagnosed with T-ALL at five months (Figure 1). The median survival of patients with APL could not be calculated, as few patients diagnosed with APL died during the period of this study. Of the patients diagnosed with ABL, one died nine days after diagnosis and another was alive until the end of this study.

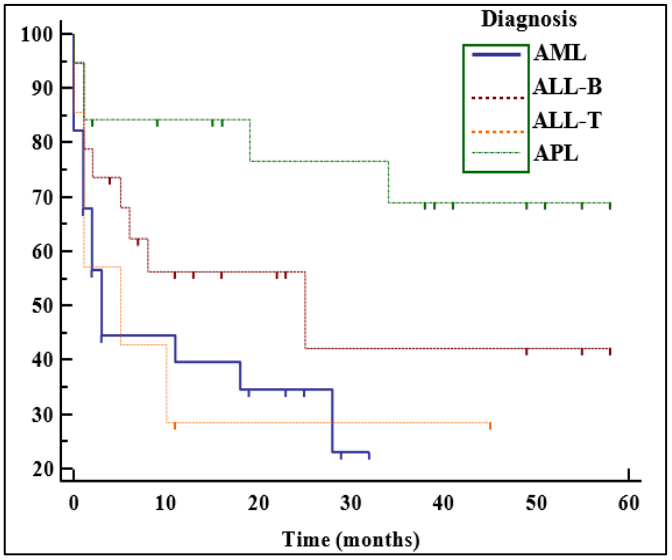

Figure 1: Survival curve of patients diagnosed with acute leukemia.

Table 3 : Clinical and laboratory characteristics of patients diagnosed with acute leukemia at the time of diagnosis, according to response to the first cycle of induction therapy.

	Negative MRD (n=25)	Positive MRD (n=25)	Total (n=50)
parameters	No. %	No. %	No. %
Age (years)			
median	35	54*	41
Limits	15-76	29-87	15-87
Leukometry (x10^3/µL)			
median	3.9	10.7	5.7
Limits	0.3-177.2	0.6-288.7	0.3-288.7
Blasts (%)			
median	59.1	52	56.1
Limits	6.0-98.8	5.4-96.0	5.4-98.8
Lactate dehydrogenase(U/L)			
median	308.5	297	308.5

Limits	0.0-3060.0		131.0-3197.0		0.0-3197.0	
LA subtype						
AML	20	35.4	11	55.2	29	39.2
EPS	19	33.5	2	11	20	26.6
ALL-B	16	27.6	5	27.6	20	26.6
ALL-T	6	11.8	1	5.6	7	9.8
ABL	1	2	1	5.6	2	2.9
Genre						
Male	36	63	8	44.2	42	56
Female	26	47.3	12	60.8	37	49
FMS3-DIT	0		0		0	
negatives	43	76.8	11	55.2	51	68.6
positives	2	4	1	5.6	3	4.2
Unrealized	17	29.5	8	44.2	24	32.2
FMS3-D835						
negatives	38	66.9	12	60.8	47	63
positives	4	7.9	0	0	4	5.6
Unrealized	20	35.5	8	44.2	27	36.4
survivin						
negatives	48	86.6	15	77.4	61	81.2
positives	5	9.8	1	5.6	6	8.4
Unrealized	7	13.8	4	22.1	12	15.4

MRD – Minimal residual disease; AL – Acute Leukemia; AML – Acute Myeloid Leukemia; APL – Acute Promyelocytic Leukemia with TL-5; ALL-B – Acute Lymphoblastic Leukemia type B; T-ALL – T-type Acute Lymphoid Leukemia; ABL – Acute Biphenotypic Leukemia; LDH – Lactate Dehydrogenase. Mann-Whitney U Test, Chi-square Test or Fisher's Exact Test. *Statistical significance when compared with the negative MRD group, P<0.05.

Expression of MDR Proteins

Of the 50 patients included in this study, 30 had the expression of MDR proteins evaluated at the time of diagnosis. The other patients could not be analysed due to the insufficient amount of sample. As performed for the evaluation of gene transcription, the patients were divided into five groups: AML (n=18), APL (n=14), ALL-B (n=9), ALL-T (n=4) and ABL (n=1). As previously mentioned, this division is due to the differences in prognosis that these AL subtypes present. As can be seen in Table 4, the medians (limits) of expression of ABCB1, ABCC1 and LRP proteins were 2.21 (0.00-116.15), 1.14 (0.00-7.54) and 0.59 (0.00-39.93), respectively. There was no correlation between the intensity of expression of the different MDR proteins.

Next, we evaluated whether there was an association between the expression of MDR proteins and the response to treatment (Table 4). In patients with AML, there was an association between the highest expression of ABCC1 and the presence of MRD (P=0.017). When assessing whether there was an association between the differential diagnosis and the expression of proteins, it was observed that the highest expression of LRP was associated with the diagnosis of AML (P=0.025). This association also remained significant for the group of patients diagnosed with AML who went into remission (P=0.008).

Table 4: Median expression of ABCB1, ABCC1 and LRP in samples from patients with acute leukemia at diagnosis, according to response to the first cycle of induction therapy and differential diagnosis.

	Protein	Negative MRD (n=15)	Positive MRD (n=15)	Total (n=30)
TOTAL of AL (n=46)	ABCB1			
	median	2.09	2.74	2.21
	Limits	0.00-116.15	0.87-83.70	0.00-116.15
	ABCC1			
	median	1.12	1.37	1.14
	Limits	0.00-7.54	0.75-3.02	0.00-7.54
	LRP			
	median	0.8	0.13	0.59
	Limits	0.00-39.93	0.00-11.40	0.00-39.93

AML(n=18)	ABCB1			
	median	1.86	15.9	3.13
	Limits	0.00-10.77	1.48-83.70	0.00-83.70
	ABCC1			
	median	1.07	2.02*	1.14
	Limits	0.00-1.57	1.00-2.02	0.00-3.02
	LRP			
	median	8.51	0.13	6
	Limits	0.00-39.93	0.00-11.40	0.00-39.93
APL(n=14)	ABCB1			
	median	2.64	3.54	2.64
	Limits	1.42-29.46	0.87-6.20	0.87-29.46
	ABCC1			
	median	1.15	0.99	1.15
	Limits	0.61-5.64	0.75-1.23	0.61-5.64
	LRP			
	median	0.21	1.4	0.32
	Limits	0.00-2.48	0.00-2.79	0.00-2.79
ALL B(n=9)	ABCB1			
	median	3.04	1.78	1.78
	Limits	1.21-116.15	1.51-14.51	1.21-116.15
	ABCC1			
	median	1.18	1.37	1.32
	Limits	0.88-7.54	0.91-1.40	0.88-7.54
	LRP			
	median	0.5	1.3	0.5
	Limits	0.00-6.74	0.00-2.61	0.00-6.74
ALL T(n=4)	ABCB1			
	median	1.51	2.02	1.76
	Limits	1.19-4.99	-	1.19-4.99
	ABCC1			
	median	1.05	1.24	1.14
	Limits	0.65-1.74	-	0.65-1.74
	LRP			
	median	0	0	0.26
	Limits	0.00-1.17	-	0.00-1.17
ABL(n=1)	ABCB1			
	median	-	2.12	2.12
	Limits	-	-	-
	ABCC1			
	median	-	1.08	1.08
	Limits	-	-	-
	LRP			
	median	-	0	0
	Limits	-	-	-

*Statistical significance when compared with the negative MRD group, P<0.05.

Discussion

Clinical and Laboratory Characteristics of Patients

ALs constitutes a heterogeneous group of malignant neoplasms that affect individuals in all age groups and are slightly more frequent in men. As expected, the group of patients included in the present study showed a predominance of males (n=25; Female=25) and a wide age range (15-87

years; Table 3). In Addition, the median age of patients diagnosed with AML was 54 years (18-87 years), while that of those diagnosed with ALL was 30 years (15-60 years).
Worldwide, the median age at diagnosis of adult patients with AML and ALL is 63 to 72 years and 25 to 45 years. years, respectively. At the Iraq, a study carried out in Baghdad with AML patients found a median age at diagnosis of 43 years (n=69). Studies reported median age at diagnosis in AMLs of 34 years (n=109), 67 years (n=97), 35 years (n=17) and 34 years (n =115), respectively. Regarding ALLs, a study in Bahia and another in Rio Grande do Sul reported medians of age at diagnosis of 49 years (n=70) and 26 years (n=42), respectively. As can be seen, with the exception of the Baghdad study, the patients diagnosed with AML evaluated here had a median age slightly higher than that found in other studies (James, 2017), but still lower than that reported in international studies. On the other hand, the median age of patients diagnosed with ALL included in the present study was similar to those reported in national and international studies.
Unlike the other AL subtypes, APLs do not show an age-peak distribution. Its incidence progressively increases during adolescence, reaches a plateau during early adulthood and remains constant until it decreases around the age of 60. Thus, most APL diagnoses occur between 20 and 50 years of age. Therefore, the median age (26 years) and age group (15-87 years) of patients diagnosed with APL included in the present study are in line with expectations for this AML subtype.
Still regarding the age of the patients included in this study, it was found that individuals who did not go into remission after induction therapy had a significantly higher median age (54 years) than patients who went into remission (35 years; P< 0.001; Table 3). As already mentioned, “advanced age” is considered a poor prognostic factor independent of ALs. In addition, older patients generally have a higher occurrence of other poor prognostic factors, such as unfavorable cytogenetic abnormalities, greater susceptibility to the toxic effects of chemotherapy, overexpression of genes associated with MDR, and the presence of contraindications to intensive care, which explains because these patients usually do not respond as well to chemotherapy. Therefore, it was already expected that, in the present study, patients with positive MRD would present a median age higher than those who entered complete remission after induction therapy.

In the present study, the group of patients who went into remission and the group that presented MRD after induction therapy had similar median leukocyte counts of 3,900 leukocytes/mm3 and 10,700 leukocytes/mm^3, respectively (P>0.05; Table 3). Iraq study carried out in Abdulsalam, et al. (2010) reported a median number of leukocytes a little higher (12,600 leukocytes/mm^3; n=115) than that found in this study. However, a study carried by Liu, (2002) in terms of ethnic and socioeconomic characteristics, reported that 51% of the evaluated patients had leukocyte counts below 10,000 leukocytes/mm^3 (n= 39), which is very similar to the leukometries found in the present work. In addition, an interesting fact observed is that both the good prognosis group (negative MRD) and the poor prognostic group (positive MRD) had medians of leukometry considered as having a good prognosis and within the reference values stipulated by the Clinical Analysis Service BTH (3,800 to11,000 leukocytes/mm^3). However, it is important to note that of the 19 patients who did not go into remission after induction therapy, seven (36.8%) had a WBC greater than 30,000 leukocytes/mm3, while in the group with negative MRD, only ten patients (17.8%) out of a total of 56 had a leukocyte count above 30,000 leukocytes/mm3. Thus, it is evident that, despite the leukocyte medians of the two groups being within the parameters of a good prognosis, the group with positive MRD presented, as expected, a higher frequency of patients with leukocyte counts greater than 30,000 leukocytes/mm^3. Another fact to be highlighted is that, although the median of the leukometry of the patients who went into remission (3,900 leukocytes/mm3; Table 3) was within the reference limits, it is very close to the lower limit of normality adopted for this parameter by the Clinical Analysis Service of BTH(3,800 leukocytes/mm3). Probably, the low leukocyte count is due to the high number of APL cases in the negative MRD group (n=17). This AML subtype usually has a favorable prognosis and is characterized by frequently presenting pancytopenia at diagnosis.

As with the leukocyte count, the median percentage of blasts at diagnosis was similar between the two groups of patients (negative MRD – 58.4% and positive MRD – 51.8%; P>0.05; Table 3). As mentioned earlier, morphological discrimination between leukemic blasts and non-leukemic atypical mononuclear cells is difficult, which implies the use of absolute leukocyte counts and not blasts as a prognostic factor. In this study, the percentage of blasts at diagnosis was obtained from the results of immunophenotyping, which eliminated the possibility of confusion between atypical mononuclear cells and blasts. However, the immunophenotyping performed for the diagnosis of ALs in this study was qualitative and not quantitative. Qualitative methods only provide relative cell counts, which makes it impossible to calculate the absolute number of blasts in the sample and therefore, unlike the absolute leukocyte count, does not provide a true notion of the tumor mass. Thus, the usefulness of the percentage of blasts at diagnosis as a prognostic parameter is quite limited. Thus, it is expected that in this study it was not possible to establish an association between the percentage of blasts and the response to treatment.

LDH is an intracellular enzyme whose activity is associated with cellular metabolism, which makes it a marker of cell proliferation and cell injury. Thus, the increase in serum LDH is a reflection of the great proliferative capacity of neoplastic cells in patients with AL and is considered a poor prognostic factor. The present study found no difference between the LDH medians of patients with negative and positive MRD (308.5 U/L and 297.0 U/L, respectively; P>0.05; Table 3). When compared separately, cases of AML with negative and positive MRD (275.0 U/L and 268.0 U/L, respectively; P=0.876) and ALL with negative and positive MRD (334.5 U/L and 610.0 U/L, respectively; P=0.247) also showed no significant differences between them. Some studies (Viana,2001) report that the elevation of LDH appears to be related to worse overall survival, lower rate of complete remission, and higher probability of premature death and resistance to treatment in cases of AML. However, like the present study, other studies (Bonilla, 2010) also found no association between LDH and the prognosis of patients

diagnosed with AML. In fact, the relationship between LDH and the prognosis of AMLs is not fully established, and the protocols for diagnosis and treatment of AMLs still do not include LDH as a prognostic factor. On the other hand, in ALLs in general, the elevation of LDH does not seem to be related to complete remission rates, but with the risk of CNS infiltration and, more specifically in T-ALL cases, with the worst overall survival. Thus, the results found in the present study are in agreement with studies (Medeiros, 2019) that report that LDH does not influence the response to induction therapy of AML and ALL patients. However, it should be noted that LDH is a nonspecific marker of tissue injury and can be altered in different situations, such as infections, acute pancreatitis and hemolytic anemias. Many of the patients included in the present study had concomitant diseases that could affect LDH levels, which may have influenced the analysis of the impact of LDH on the prognosis of patients included in the present study.

Regarding the presence of FMS3-DIT, of the 50 patients evaluated for the presence of this mutation, only three (5.8%) had it, and of these, two (3.9%) were diagnosed with AML (one one case of APL and another of AML without maturation) and one (1.9%) of ALL (ALL-B of the common type). The frequency of patients diagnosed with ALL and carriers of the FMS3-DIT mutation found in the present study is in line with what is expected for cases of ALL, which is 0% to 10%. However, interestingly, the frequency of FMS3-DIT in cases of AML is lower than that found in international and national studies, which report an incidence of FMS3-DIT of 12% to 32% in AMLs. Furthermore, FMS3-DIT is commonly associated with TL-5 and is present in approximately 35% of APL cases. That makes this low frequency even more unusual, since of the 50 samples analysed, 13 were from patients who had TL-5. This low incidence of FMS3-DIT in cases of AML may be related to intrinsic characteristics of the population studied. However, there is a possibility that the incidence reported by other studies (Medeiros, 2019) is overestimated. This overestimation stems from the fact that many of these studies are retrospective and use stored samples. The stored samples usually belong to patients who had leukocytosis at the time of diagnosis and, therefore, had excess cells that could be stored. Elevated leukocyte counts at the time of diagnosis are known to be associated with the presence of mutations in the FMS3 gene. Thus, the use of these stored samples by retrospective studies may have led to an overestimation of reported incidences. Of the three cases that presented the FMS3-DIT mutation (n=3), the patient diagnosed with ALL-B of the common type (51 years old) presented with significant leukocytosis (288,680 leukocytes/mm^3) and died one month later. diagnosis without ever going into remission. The patient diagnosed with AML without maturation (57 years old) also had an AL condition with an elevated WBC count (19,310 WBC/mm3), but went into remission after induction therapy and underwent an allogeneic OM transplant. However, one year after his diagnosis he had a recurrence and died. The third case that presented FMS3-DIT was a patient with APL (23 years old) who had leukocytosis (44,600 leukocytes/mm^3), immunophenotypic markers of poor prognosis (CD34+/++ and CD2-/+) at the time of diagnosis. and, in the search for TL-5, the short isoform (bcr3 – breakpoint site 3) of the translocation. This patient went into remission after induction therapy, but had a relapse about two years after his initial diagnosis. He underwent a new chemotherapy treatment and an autologous BM transplant and is currently in remission.

Conclusion

The results of the tests are used to make the diagnosis. Anemia and thrombocytopenia are virtually usually present in some form or another in the patient. A lower, normal, or higher total leukocyte count is possible (Blackburn, 2019). The presence of blast cells on a blood smear is a given unless the white blood cell count is significantly reduced. Bone marrow testing should always be done in addition to a blood smear for definitive diagnosis. Occasionally, bone marrow aspirates yield such a hypocellular specimen that a needle biopsy is required. Examination of the bone marrow biopsy should include cytological study of the imprints and sections for cellularity. Aplastic anemia should be included in the differential diagnosis of severe pancytopenias, but bone marrow biopsy should be definitive. On the other hand, an experienced observer will not mistake the atypical lymphocytes of infectious mononucleosis for leukemic cells. It is important to differentiate ALL blasts from LNLA blasts. In addition to smears with the usual stains, the use of the following histochemical stains is often helpful: para-aminosalicylic acid (PAS), myeloperoxidase, Sudan black B, and nonspecific esterases.

Acknowledgements

I extend my sincere thanks to everyone who contributed to the completion of the paper project, especially to them Department of Biology, College of Education, University of Tikrit ,General Directorate of Education Salah Al-din and University of Çankırı Karatekin Department of Biology , Molecular biology.

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